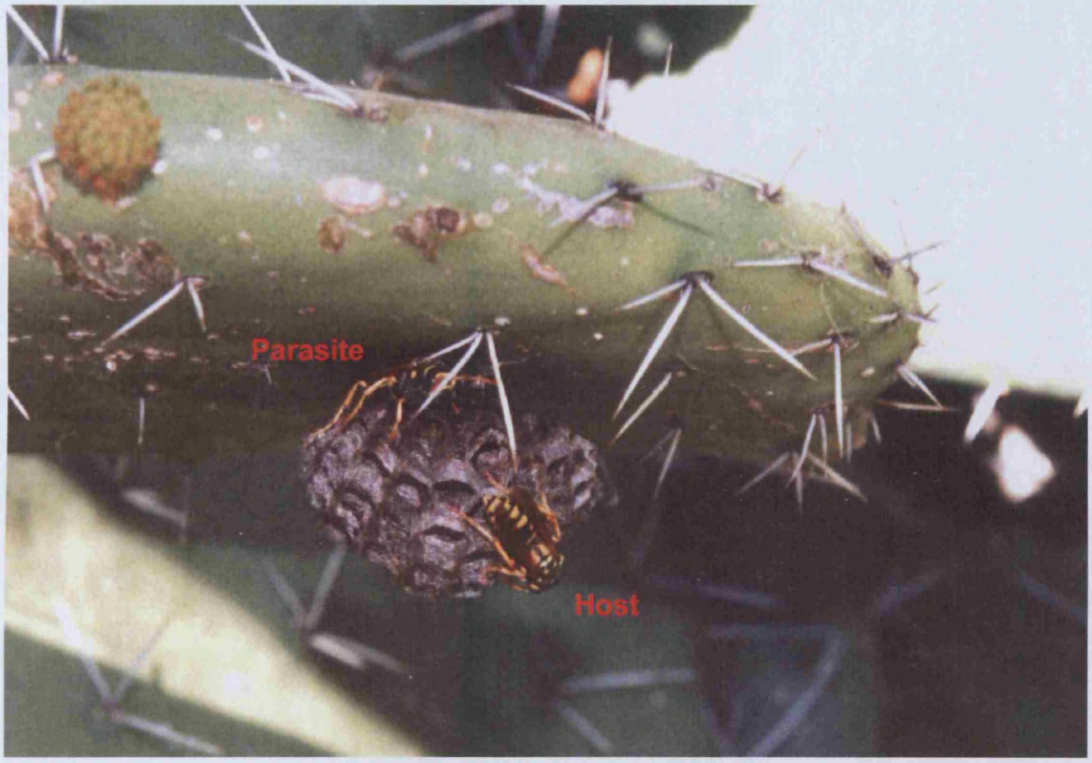
UNIVERSITY OF LONDON THESIS

Degree PhD Year 2007 Name of Author ALMOND, Edward James

Interaction between the paper wasp *Polistes dominulus* and its social parasite, *Polistes semenowi*



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Submitted for Examination of PhD Degree
January 2007

I, Edward James Almond, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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I'd rather be in Tokyo
I'd rather listen to Thin Lizzy-oh
And watch the Sunday gang in Harajuku
There's something wrong with me, I'm a cuckoo

Belle and Sebastian – I'm a Cuckoo

Acknowledgements

First of all, I would like to thank Dr. Jeremy Field for all his valuable support during the production of this thesis, who as my supervisor introduced me to the wonderful world of wasps and helped me hone my scientific method. I would also like to thank Prof. Andrew Pomiankowski, my second supervisor, for his useful comments and advice.

My parents, Jack and Brenda, inspired me at an early age with their spirited love of nature and have continued to nurture my own interest throughout my life. For them, words could never be enough, but I thank them with all the love I have for what they have done for me. My grandparents also inspired me, I still recall days walking in the Dales or being given fruits (and wines!) of their labours. Their understanding of the natural world around them truly astounded me.

I would like to thank the entire University College London Biology department for their sterling support over the years of my studies. Lorenzo Zanette was a diligent aid in Spain, keeping me going with his morale-boosting tales and ruses. Dr. Catherine Bridge provided such a good example in her diligence in field work and good nature throughout the struggle of her own endeavours, she is a rare beacon of humanity and humility that never failed to shine for me. Ian Evans was a fellow adventurer in the London music scene who would valiantly attend even the most hardcore Latvian Progressive-metal gig. I often wonder whether my attendance at Curry Fridays affected my work rate, but the sparkling minds of Eric and Lewis no doubt fired the imagination, if not the keyboard fingers post-feast. When dark thoughts distracted, the ever ready Ian, Duncan, Sam and Callum provided both an outlet and sound thrashing in the Quake arena. The "Darwinites", ever present at post-graduate events, amused me thoroughly with their antics.

In Andalucia, the wonderful Shawna welcomed me into her home and enticed me into her bar with fabled medicinal liquors which aided me in my midnight jaunts to the cactus. From Cambridge, the "Phoenix Clique" remained alit long since we all (mostly) left university, their random postings always a welcome distraction. My flatmates Tom, Jon and Tat too provided respite with good whisky and disturbing conversation.

Finally, I'd like to thank Jen, a muse, scholar, friend and insatiable Marmite addict who infused me with such enthusiasm (no doubt obtained from all those yeast-based B vitamins) that even statistical analysis could not dull.

Abstract

Polistes dominulus is a social paper wasp found around the Mediterranean basin and in North America. Queens of the social parasite *Polistes semenowi* invade the nests of *P. dominulus* and raise parasite offspring by exploiting the foraging effort and nest defence provided by host wasps. Recent studies have suggested that nest infiltration by the parasite involves "hacking" into the host's nestmate recognition system, so that hosts accept the parasite as one of their own. However, this mechanism is employed only after an initial violent attack upon the nest, which appears to be resisted by hosts. It is therefore unclear whether *P. dominulus* females are truly deceived by the parasite. The aim of this thesis is to investigate possible strategies that hosts can adopt when faced with parasite attack, if they are not completely deceived by the parasite's subterfuge.

An obvious host counter-strategy is simply to abandon the nest altogether when attacked by a parasite, then pursue other reproductive options such as joining another host nest or re-nesting. I investigate whether host adults do indeed abandon, and what choices abandoning hosts have.

Hosts that stay on a parasitized nest may still directly or indirectly gain fitness by rearing a reduced number of host offspring. I investigate this possibility using a combination of video recording of offspring feeding in the field, and microsatellite analysis to determine offspring parentage. In particular, I focus on (1) whether hosts can still lay eggs after parasite invasion, (2) differential provisioning: the possibility that host adults feed related offspring in preference to offspring of the parasite. This is examined both in the presence and absence of the parasite adult. I also compare host helping effort and aggression levels on parasitized and unparasitized nests.

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Chapter 1. **Introduction**

1.1 Biological Interactions

Within an ecosystem, organisms interact, affecting their own fitness and the fitness of those around them (Table 1-1):

Effect on fitness of actor	Effect on fitness of recipient		
	Negative	Neutral	Positive
Negative	Competition	Amensalism	Altruism
Neutral	Amensalism	Neutralism	Commensalism
Positive	Predation or *Parasitism*	Commensalism	Mutualism

Table 1-1: A table detailing the different biological interactions that occur in an ecosystem, in terms of fitness effects on the actor and recipient of the interaction.

1.2 Parasite-Host Interaction

This thesis is concerned with **parasitism** (see shaded box in Table 1-1), when an actor (**parasite**) increases its own fitness at a detriment to the recipient organism (**host**). Parasitism usually occurs over a period of time, with the parasite using host resources at the expense of the host. With predation, the actor kills the host immediately, so obtains only the resources the host has at the time of the kill. With parasitism, the parasite often keeps the host alive in order to exploit host resource collection after the parasitism has commenced. Parasites also typically only have narrow range of hosts, often they rely on a single host species. Predators tend to prey on several species and in general are not specialised. There are, however, many exceptions to these generalisations.

Parasitism can be **intraspecific**, where both parasite and host are the same species, or **interspecific**, where the two are different species. Intraspecific parasitism can be anything from a *Polistes* wasp eating the egg of a reproductive rival (Field 1992) to full fledged nest takeover (Makino and Sayama 1991). There

are many interspecific parasites of *Polistes*; examples include species of Strepsiptera (e.g. *Xenos peckii*), Ichneumonids (e.g. *Pachysomoides fulvus*), Pyralids (e.g. *Chalcoela pegasalis*) and Eulophids (e.g. *Elasmus polistis*). This thesis is concerned with the interspecific parasite *Polistes semenowi*.

Parasitism may be opportunistic (or **facultative**), where parasitism occurs if an opportunity to parasitize arises but the actor in question does not need to parasitize in order to produce offspring, or **obligatory**, where the parasite relies on parasitizing the host in some part of its life cycle in order to pass on its genes. *P. semenowi* is an obligatory parasite.

1.2.a Host-Parasite Co-evolution

A parasite, by imposing a cost upon its host by reducing its fitness, creates a selective pressure for adaptations against parasitism. Should counter-parasite adaptations arise, the parasite must now develop counter adaptations itself. This creates a potentially never ending cycle of escalating countermeasures, comparable to that of nuclear stock-piling in the Cold War era (hence the popular analogy of an "evolutionary arms race"). The cycle can be broken, if the parasite switches host or either species goes extinct due to the other developing a successful countermeasure. The relative costs and benefits of host counter-parasite behaviour depend much upon the level of parasitism in the population, under low levels hosts may fair better by not displaying such behaviours as the cost of using them in error may outweigh the benefits of deploying them on the rare occasion they are parasitised (Davies 2000). A simple schematic of such a cycle is shown in Figure 1.2-1

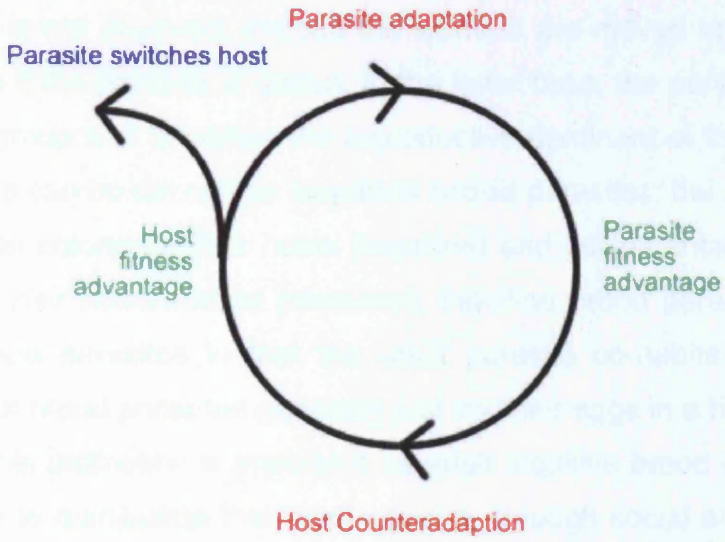

Figure 1.2-1: A schematic of host-parasite co-evolution.

In other words, parasites and hosts are continually adapting to each other, they co-evolve. When looking at host-parasite systems, it is therefore useful not only to look at parasite adaptations to exploiting the host, but also look at facets of the host that may be involved in resisting parasitism. Host-parasite co-evolution is discussed in more detail in chapter 5.

1.2.b Social Parasitism

A wide definition of social parasites includes any species that take advantage of social interactions between individuals within a social species in order to increase their fitness. This often means manipulating multiple individual organisms within a social group. Kleptoparasitism, stealing resources from a host, is included in this wider definition, although non-social species can also be hosts to this form of parasitism (Table 1-2). All social parasites can be seen as stealing resources and are hence kleptoparasites.

In this thesis, I define a social parasite as a species that specifically exploits the social structure of a social species for its own gain (Wilson 1971). Social parasitism in this sense can range from theft of host worker brood to serve as slaves in the parasite's colony to completely taking over the group and using them as a workforce through whom the parasites own offspring are reared. In all cases, the parasite infiltrates the social group and exploits the relationship between the reproductive individual and its workers. In the former case, the

original queen is not deposed; instead the workers are moved to the parasite's nest but act as if the parasite is queen. In the latter case, the parasite does take over the host group and becomes the reproductive dominant of the host colony. The latter group can be defined as **inquiline brood** parasites; the adult parasites live in the same colony as their hosts (inquiline) and get parental care for their offspring from their hosts (brood parasites). Inquiline brood parasites therefore differ from brood parasites in that the adult parasite co-habits with the host whereas normal brood parasites generally just lay their eggs in a host colony and do not stay. This distinction is important as adult inquiline brood parasites have the opportunity to manipulate their hosts directly through social actions whereas brood parasites rely on adaptations in their offspring to manipulate the host (Cervo and Dani 1994; Kilner et al. 1999).

This thesis is concerned with the social parasite *P. semenowi*, an **inquiline brood parasite**.

Type of Parasitism	Description	Intraspecific Examples	Interspecific Examples
Kleptoparasitism	Theft of food or other resources	Oystercatchers (Tuckwell and Nol 1997), Whelks (Ishida 2004)	Spiders (Cangialosi 1990), Gall thrips (Crespi and Abbot 1999), *Sphecodes* bees (Sick et al. 1994)
Slave-making	Enslavement of host brood	Humans (Thomas 1997), *Polyergus rufescens* (Lemoli et al. 1993)	*Polyergus rufescens* (Mori et al. 1995), *Formica sanguinea* (Mori et al. 2001)
Brood parasitism	Lack a worker caste, do not build their own nest, lays eggs in host nests, exploiting it to raise parasite offspring	Maned ducks (Briggs 1991), Fish (DeWoody and Avise 2001), Nest usurpation in *Polistes* (Cervo and Lorenzi 1996)	Some Cowbirds and Cuckoos (Davies 2000), Ants (Johnson et al. 1996), ***Polistes semenowi*** (Mead 1991)

Table 1-2: A summary of the main types of social parasitism.

1.2.c Interspecific Social Parasite Evolution

Emery noted that social parasites were in many cases closely related to the species which they parasitised (Emery 1909). This suggests **sympatric** speciation, where a new species evolves from another within the same habitat; there is no physical barrier to gene flow. In many cases the phylogenetic relationship between social parasite and host is close (Wilson 1971; Holldobler and Wilson 1990) suggesting sympatric speciation. Despite not being geographically isolated, some authors suggest that use of different sites for mating may aid this speciation. (Wilson 1971; Holldobler and Wilson 1990; Turillazzi et al. 1993) The use of a communal mating site allows individuals of relatively rare species to reliably find conspecifics to mate with.

An alternate route to social parasitism involves **allopatric** speciation, where a physical barrier stops gene flow and the two populations of one species diverge into two reproductively isolated species. Should one of the two new species become a social parasite, it may further diverge into several species locally adapted to their specific hosts, such as with *Vidua* finches (Sorenson and Payne 2001). This hypothesis therefore predicts that social parasites within a genus would form a monophyletic group and be more closely related to each other than to their hosts.

1.3 Interspecific Obligate Brood Parasites

An obligate inquiline brood parasite is defined by its total dependence upon the host species in order to rear its offspring. Obligate brood parasites are mostly concentrated within the hymenoptera and birds. A review of all hymenopteran and avian obligate brood parasites is not given here; I focus solely on the family Vespidae in order to show the evolutionary context of *Polistes* social parasites. A comparison of *P. semenowi* and avian brood parasites is given later in this chapter.

1.3.a Social Parasitism within the Vespidae

Within the social wasps of the family Vespidae, of the eusocial subfamilies Stenogastrinae, Polistinae and Vespinae, only the latter two contain described social parasites (Figure 1.3-1):

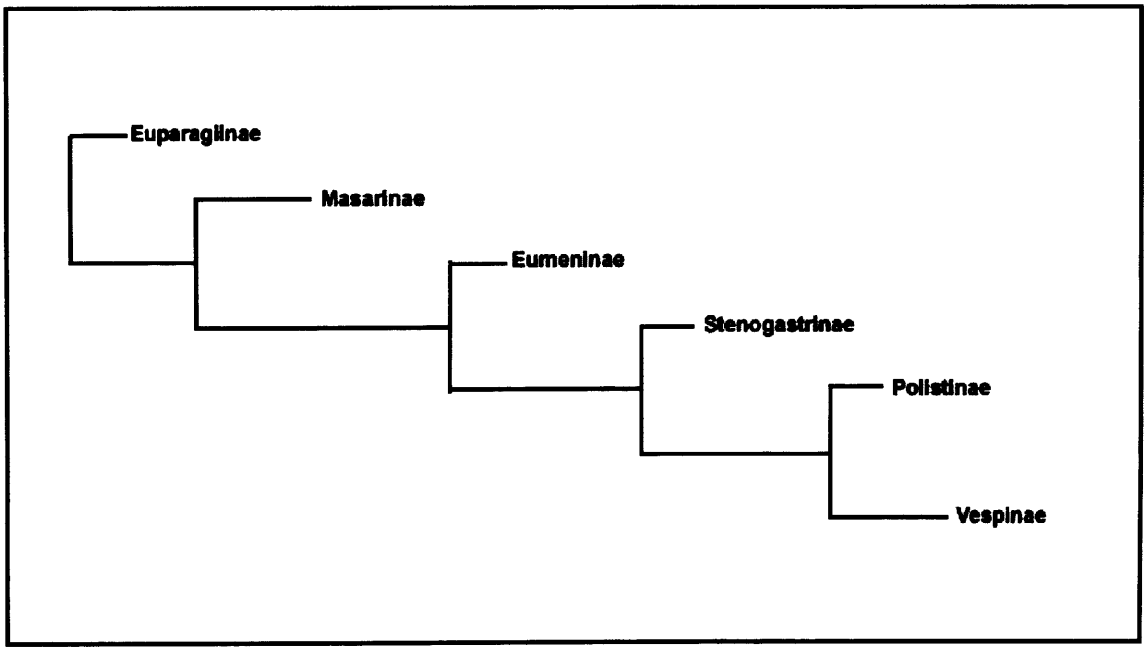

Figure 1.3-1: Cladogram of the subfamilies of Vespidae (Carpenter 1982), from Ross and Mathews 1996.

1.3.a.i. Vespinae

Within the Vespinae, there are three known species of inquiline brood parasite; *Vespula austriaca*, *Dolichovespula omissa* and *D. adulterine* (Carpenter and Perera 2006). These species are not more closely related to their host species than other non-host species and so Emery's Rule does not seem to apply (Carpenter and Perera 2006). The two *Dolichovespula* species are more closely related to each other than their hosts, suggesting allopatric speciation. All three parasitic species infiltrate host nests and take over the reproductive dominant position. They lack a worker caste and rely on host workers to forage and provision their young.

1.3.a.ii. Polistinae

Polistes sensu strictu is the only genus within the Polistinae to have social parasites (Arevalo et al. 2004). There are three obligate brood parasite species within this genus; *P. semenowi*, *P. atrimandibularis* and *P. sulcifer*. This thesis concentrates upon the parasitism of *P. dominulus* nests by *P. semenowi*.

The evolutionary relationship between the three socially parasitic species of *Polistes* and their hosts has been examined by analysing 16s rRNA gene sequences as a basis for constructing possible evolutionary trees (Choudhary et al. 1994). The most statistically robust trees (using maximum likelihood) indicated that the three social parasite species form a monophyletic group (Figure 1.3-2) and that this group is closely related to their hosts. This close phylogenetic relatedness may be a major factor in the ability of *P. semenowi* to invade *P. dominulus* colonies, as closely related species are probably more likely to have similar morphological, behavioural and chemical features.

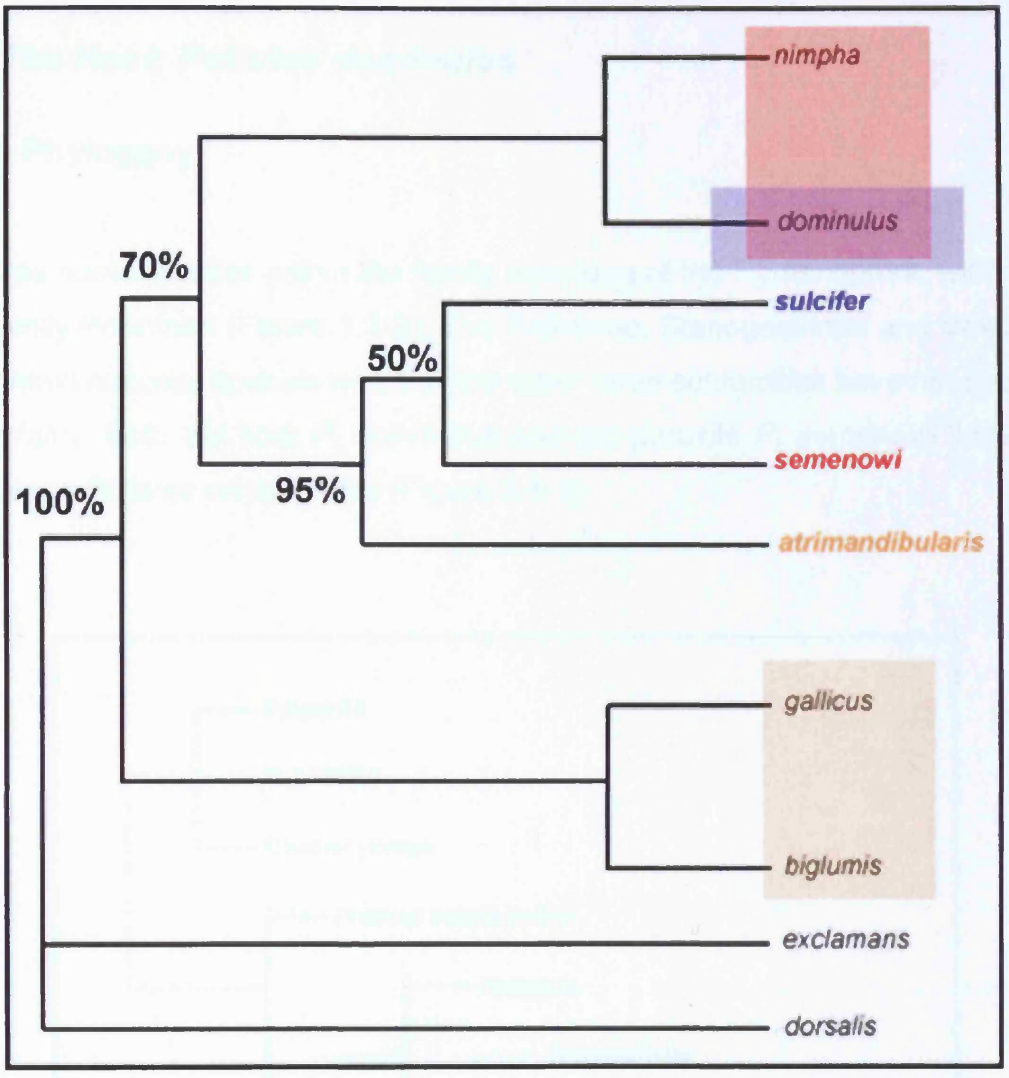

Figure 1.3-2: Phylogeny of *Polistes sensu strictu* (Choudhary et al. 1994), parasite species are in bold colour, the coloured boxes indicating which host species the corresponding parasite attacks. Confidence of branches calculated using Maximum Likelihood are reported.

1.4 The Host; Polistes dominulus

1.4.a Phylogeny

Polistes dominulus lies within the family Vespidae of the Hymenoptera, within the subfamily Polistinae (Figure 1.3-2). The Polistinae, Stenogastrinae and Vespinae all contain eusocial species whereas the other three subfamilies have not evolved eusociality. Both the host *P. dominulus* and the parasite *P. semenowi* lie within the genus *Polistes sensu strictu* (Figure 1.4-1).

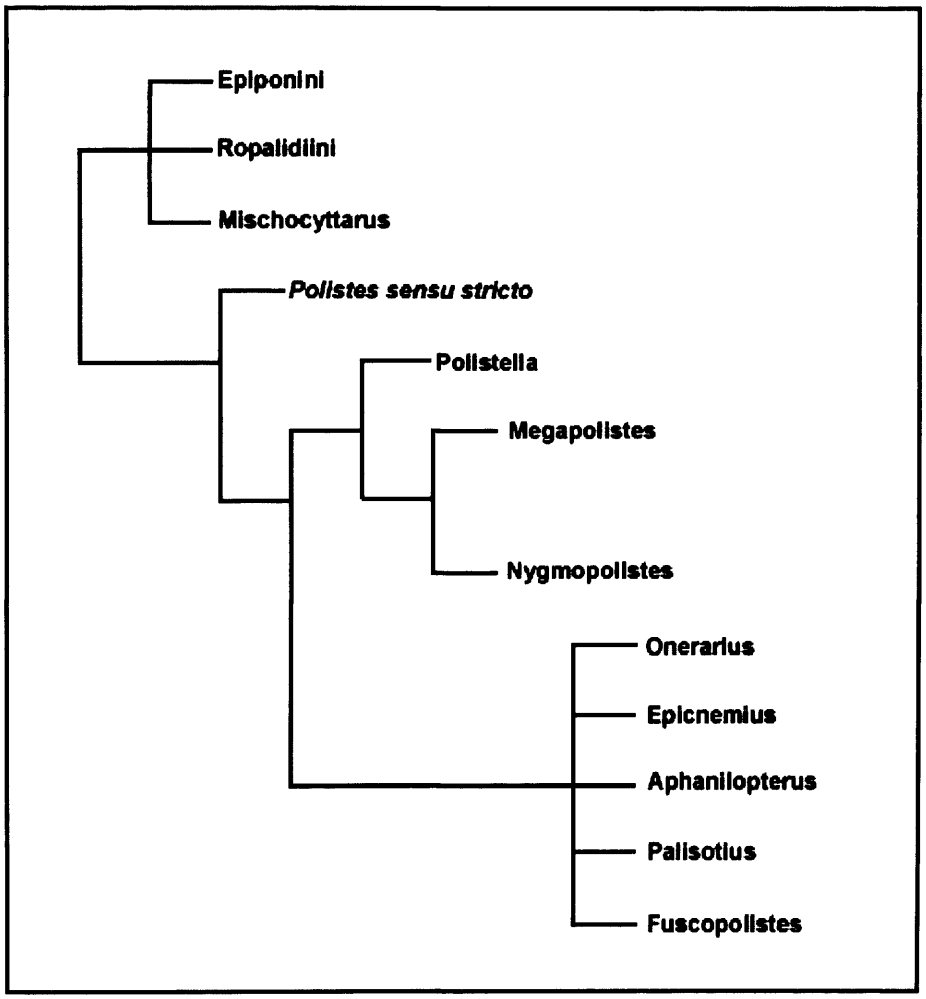

Figure 1.4-1: Cladogram of the genera of Polistinae (Arevalo et al. 2004)

1.4.b Distribution

Polistes dominulus is distributed mainly in the Palaearctic Region, particularly around the Mediterranean basin, with recent introductions as an invasive species in North America (Massachusetts) and Australia (Figure 1.4-2).

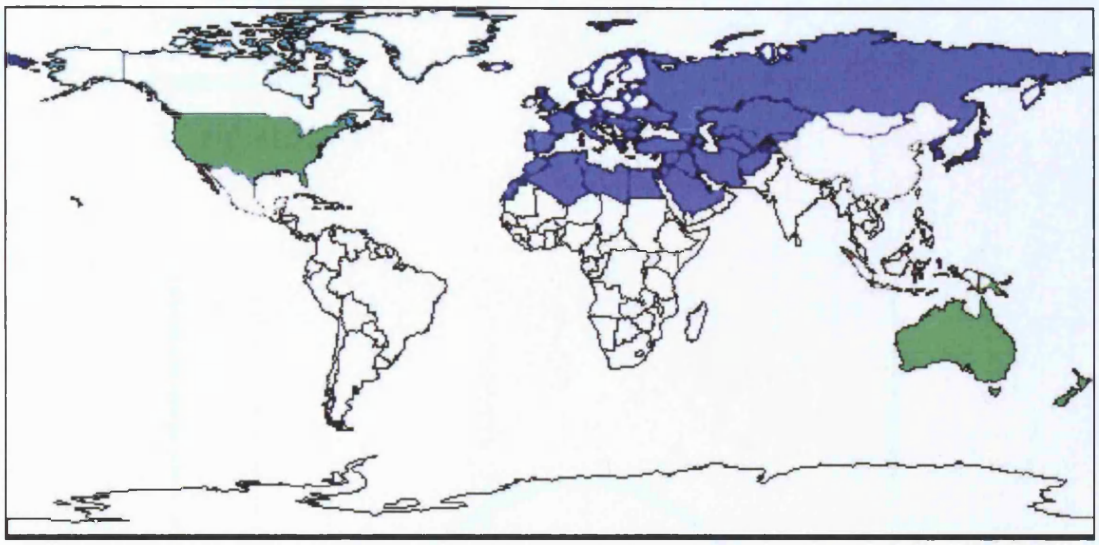

Figure 1.4-2: A map showing the known distribution of *Polistes dominulus*. Areas in blue are native range, green areas are where human activity has introduced it as an invasive species.

1.4.c Habitat

P. dominulus nests can be found wherever a suitable substrate to attach the nest occurs. This can be on the stems of slow growing plants, human structures such as houses or fence posts or naturally occurring structures such as rocks. In my field sites in Conil, the majority are found on *Opuntia* cactus, more details are given in chapter 2.

1.4.d Colony Cycle

The nesting cycle of *P. dominulus* can be divided into four main phases (Reeve 1991) as shown in Figure 1.4-3:

1. Founding
2. Worker
3. Reproductive
4. "Intermediate"

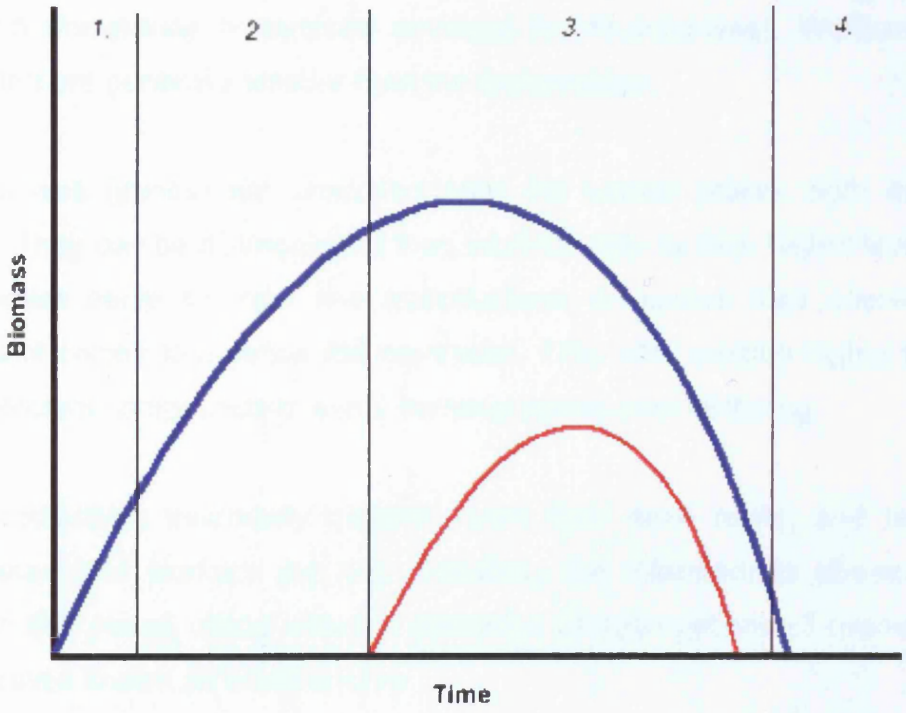

Figure 1.4-3: A qualitative depiction of *P. dominulus* colony biomass, blue line depicting entire colony biomass, red line depicting solely reproductives (adapted from Ross and Mathews 1996.)

The founding phase involves a lone female (foundress) initiating a nest, building several cells before laying the first egg. She is frequently joined by other females at this stage; often these are related wasps (personal observation, but see Queller et al. 2000). Depending on climatic conditions, this occurs in the months of January and February at the field sites I used.

After the first eggs hatch, the foundresses feed them with malaxated insect flesh, in a process called "progressive provisioning"; the larvae is given sufficient food to develop to adulthood over a period of time, rather than all at once as with "mass provisioning". Egg-laying is stopped until some of these first larvae develop into pupae. Generally these first brood will serve as the first generation workers of the colony.

The worker phase occurs usually two months after colony initiation, the time taken to build the nest and rear these first workers to adulthood. The workers take over the majority of the colony's foraging, nest defence, brood care and nest maintenance. The workers do not, however, take over egg laying; the foundresses still retain reproductive control over the nest (see later for more details on dominance hierarchies amongst the foundresses). Workers are all female and are generally smaller than the foundresses.

Reproductives (gynes) are produced after the worker phase, both male and females. They can be distinguished from workers only by their higher levels of fat stores which serve to allow the reproductives to survive their over-wintering period until colony foundation the next year. They also contain higher levels of cryo-protectant compounds to avoid freezing during over-wintering.

The reproductives eventually disperse from their natal nests, and remaining foundresses and workers die out, signalling the intermediate phase. Mating occurs in this phase, along with the formation of aggregations of over-wintering reproductives known as hibernaculae.

1.4.e Nest Construction

Nests are constructed from pulped plant matter; at the field sites, foundresses used the brittle, dead stalks of various flowers. Foraging adults scrape the stems and mix it with mouth secretions to form the pulp.

Nests are initiated with a long stalk (pedicel) on the substrate. The foundress then builds a single cell at the end of this stalk, before building cells around it, producing the characteristic hexagonal patterning of cells. The nest is open celled (Figure 1.4-4) until the pupal stage, where larvae secrete a silk cap to cover the cell while they metamorphose into adults. The contents of cells can easily be seen and differentiated (egg or different stages of larvae) with the naked eye.

Construction of the nest is not monopolised by one individual, it consists of many individual efforts by workers and foundresses (Karsai and Theraulaz 1995). This decentralised method of nest construction was termed "stigmergy", where the nest itself provides cues as to where further construction should occur (Karsai and Penzes 1993; Karsai 1999). It has been suggested that these cues direct where, and at what rate, nest growth occurs (Karsai 1997; Karsai 1999; Karsai and Balazsi 2002).

Figure 1.4-4: A *Polistes dominulus* nest.

1.4.f A Primitively Eusocial Paper-Wasp

Polistes dominulus is a primitively eusocial species of paper wasp. A eusocial group has historically been defined as having the following characteristics (Batra 1966):

1. reproductive division of labour
2. overlap of generations
3. cooperative care of young
4. sterile castes

Genetic data suggests that there is normally only one reproductive individual on a *P.* dominulus nest at any one time (Queller et al. 2000). Non-reproductive wasps on the nest are not morphologically distinct from the reproductives of *P. dominulus.* They are seemingly either physiologically prohibited by, or refrain from laying eggs in response to a signal from, the dominant individual (Cervo and Lorenzi 1996; Sledge et al. 2004). Some that are capable of egg-laying have their eggs destroyed by the reproductive (Liebig et al. 2005). Hence, whilst having the potential to reproduce, *P. dominulus* workers and subordinates are effectively sterile through the action of the dominant individuals (Liebig et al. 2005).

1.4.g Dominance Hierarchy

In *Polistes dominulus* nests, there is one foundress which monopolises the majority of reproductive activity (Queller et al. 2000). The dominant foundress does not usually differ significantly morphologically from subordinates in the pre-worker phase, but she does differ behaviourally, dominating other individuals, laying the majority of eggs and eating the eggs of other wasps (Pardi 1948; Pardi 1948; Theraulaz et al. 1990; Theraulaz et al. 1992). This dominant rarely forages or maintains the nest, instead spending the majority of her time involved in social interactions (Theraulaz et al. 1990). Should the alpha foundress die, the second most dominant individual, the beta foundress, takes over the role and behavioural profile of the alpha foundress (Theraulaz et al. 1990). Thus, the behavioural hierarchy is not fixed, subordinate individuals are not a sterile subordinate "caste" unable to reproduce and subordinate individuals of a certain dominance rank can

vary in the behavioural profile they exhibit (Theraulaz et al. 1992; Cant et al. 2006).

The dominance behaviour of the alpha foundress has been postulated as having a repressive effect, either through directly inhibiting the physiology of subordinates or through acting as a signal which the subordinate responds to by restraining from laying eggs (Pardi 1948; Liebig et al. 2005). Ovarian condition in the dominant is also thought to play a part (Liebig et al. 2005); ovariectomized dominants in one study did not restrict egg laying in subordinates despite managing to remain the alpha dominant on the nest (Roseler and Roseler 1989).

Subordinates spend more of their time off the nest foraging for building material and food (Cant and Field 2001). This prey is usually in the form of malaxated caterpillar flesh which the subordinates feed to the brood or pass to the dominant or other subordinates in a process known as trophallaxis. Subordinates also perform the majority of nest defence, using their stings to deter attack from vertebrate predators.

1.4.h Nest-mate Recognition

It is thought that *P. dominulus* foundresses discriminate nest-mates using an acquired environmental cue. On eclosing from their pupal cells, they absorb hydrocarbons from their natal nest and use them as a template to discriminate nest members from non-nest members (Singer and Espelie 1992; Gamboa et al. 1996). Studies of *P. fuscatus* have shown that wasps isolated from the nest, treat all conspecifics as nest mates even if highly unrelated, suggesting nest odour is important in acquiring nest mate recognition cues (Shellman and Gamboa 1982). This unique hydrocarbon signature consists of both foundress-applied hydrocarbons and the plant material-based hydrocarbons from which the structure of the nest is derived (Gamboa et al. 1996).

P. dominulus dominant foundresses have been shown to have a different epicuticular hydrocarbon profile than workers (Bonavitacougourdan et al. 1991). Dominant individuals exhibit the behaviour of frequently rubbing their gasters and abdomen across the nest surface (Theraulaz et al. 1992) and are present for longer time on the nest (Cant and Field 2001), suggesting that the dominant

individual may contribute a greater proportion of hydrocarbons to the nest "odour".

1.4.i Brood Recognition

As well as discriminating between adult nestmates and non-nestmates, *Polistes* foundresses also have the opportunity to discriminate between brood laid on their nest. Studies of *Polistes* have shown that individual foundresses do not preferentially feed their own larvae over the offspring of other foundresses (Strassmann et al. 2000).

In studies of oophagy, *Polistes* foundresses were found to be able to discriminate non-nestmate eggs from their own, eating those alien eggs that were to become reproductives, yet sparing those which would eventually become workers (Lorenzi and Filippone 2000). In both cases, the eggs were shown to have differences in cuticular hydrocarbon signatures and it was hypothesised that worker destined eggs also had a different signature to reproductive destined eggs. The differential oophagy might be explained by foundresses being less acceptant of alien odours during the later, reproductive stage of the colony cycle.

In *P. dominulus* it is yet to be seen whether foundresses can distinguish 'alien' brood from their own. Some evidence from studies of *P. sulcifer* parasitism of *P. dominulus* suggests they cannot, at least under laboratory conditions with ad-libitum food (Cervo et al. 2004). Whether or not discrimination occurs in natural conditions remains to be seen. Brood and nestmate recognition is discussed further in chapter 5.

1.4.j Previous work done on *P. dominulus* in my population

There have been several previous studies on the Conil population of *P. dominulus*. Cant and Field (2001) found that lower ranked individuals spent more time off the nest and as group size increased, wasps of an equivalent rank foraged less. Shreeves et al. (2003) observed that the group size of a nest was linearly related to the total number of brood present in the nest. They also found that Assured Fitness Returns (AFRs) favour helping amongst *P. dominulus* subordinates. AFRs occur when a helper only part-rears offspring before dying, which are then reared into adulthood by surviving group members. A single nesting foundress would lose all the effort put into partially raising brood, but by nesting with other wasps, it safeguards the effort it makes should it die.

Cant and English (2006) reported a positive linear relationship with group size and the proportion of potential breeders, based on analysis of the ovarian development of foundresses. Cant et al. (2006) analysed aggression between adjacent foundresses in the dominance hierarchy and found both the rates of aggressive displays (acts aimed at lower ranking individuals) and tests (acts aimed at higher ranking individuals) decreased down the hierarchy. They also found that aggression increased as the season progressed. Finally, Cant et al. (2006) experimentally induced contests over dominance rank and discovered that the occurrence of escalating fights with the dominant increased when the subordinate had lower levels of ovarian development and with larger group productivity.

1.5 The Parasite: Polistes semenowi

Polistes semenowi is a classic social inquiline brood parasite; it lacks a worker caste and seemingly the ability to build its own nest. It relies totally on usurping nests of *P. dominulus* and exploiting these hosts to rear its young. Very little work has been done on *P. semenowi*, especially in the field.

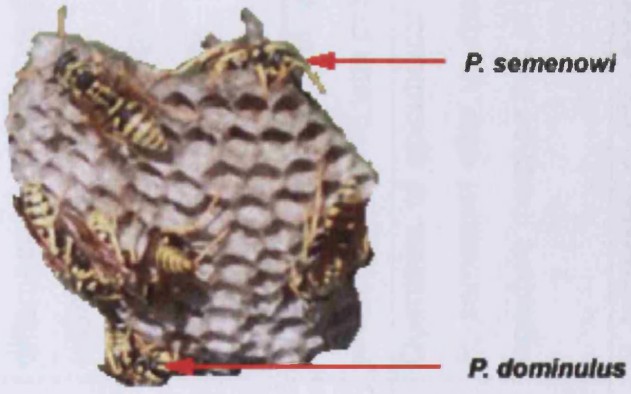

Figure 1.5-1: A parasitised *P. dominulus* nest upon *Opuntia sp.* cactus in Conil.

It has a more limited geographical distribution than the *Polistes* species it parasitizes, mainly occurring in the areas around the Mediterranean and Caspian basins (Guiglia 1972).

Work on *P. semenowi* has so far focussed on observation of its invasion tactics and on its chemical profile. A summary of this research is given in Table 1-3.

1.5.a Parasite Morphology

P. semenowi has several morphological adaptations that aid its parasitism of *P. dominulus*. Thicker, toughened mandibles and forelegs allow the parasite to easily fight off host foundresses (Carpenter et al. 1993; Zacchi et al. 1996), with a tougher exoskeleton in general aiding its defence against attack (shown in *P. sulcifer* and *P. atrimandibularis*, Cervo 1994). The sting is not modified in any way beneficial to invasion, which is consistent with it not being used in invasion (Cervo 1994).

1.5.b Summary of previous studies of *P. semenowi*

Author	Sample Size	*Biology studied*	Conclusions
Shreeves et al. 2003	21 parasitised nests (field)	Host nest choice, Parasitised brood survival, Parasitised nest survival	Parasites attack larger, more advanced nests. No effect of parasitism on brood survival. Lower abandonment rate of parasitised nests, but no difference in nest destruction or survival from unparasitised nests
Zacchi et al. 1996	12 field parasitised nests, 11 in the laboratory	Invasion behaviour	Parasite employs an aggressive usurpation tactic. Parasite strokes and licks nest surface and foundresses after taking over the nest.
Mead 1991	1 parasitised laboratory nest	Reproduction productivity of host and parasite	The hosts on the nest studied halted reproduction whilst the parasite was present.
Lorenzi et al. 2004	4 parasitised nests, 25 hibernating and 3 spring pre-usurpation parasites in the laboratory	Cuticular hydrocarbon composition of hosts, their nests and parasites before and after usurpation	Quantities of epicuticular hydrocarbons increase on *P. semenowi* after usurpation and their hydrocarbon signature matches that of their hosts
Cervo et al. 1990	1 parasitised *P. nimpha,* 2 parasitised *P. dominulus* nests in the laboratory	Invasion behaviour	*P. semenowi* invades aggressively

Table 1-3: A summary of previous studies of *P. semenowi*.

1.5.c Invasion Tactics

There have been few studies of the invasion tactics of *P. semenowi* (Table 1-3). These studies have yielded differing conclusions as to the exact nature of the parasite attack. The majority of studies suggest an aggressive strategy is followed by non-aggressive means (Cervo et al. 1990; Mead 1991; Zacchi et al. 1996). A completely non-aggressive invasion strategy was reported in a study of invasion of *Polistes nimpha* nests, another host of *P. semenowi* (Demolin and Martin 1980).

Typically, the parasite invades pre-emergence nests, initially seeking out the nests with a slow patrolling flight (Zacchi et al. 1996). The parasite is often seen near nests apparently observing foundress activity (personal observation). On landing on the nest, the parasite has to defend itself against attacks by the host foundresses, until it either drives the foundress off the nest or the host foundresses submit to it. The parasite bites and grapples with foundresses, usually having a "falling fight" where two wasps grasp each other, attempting to bite and sting, which results in the pair rolling off the nest and dropping to the ground.

One paper indicated that the parasite targets the dominant foundresses on nest attack (Zacchi et al. 1996), although this may just be a result of dominant individuals remaining on the nest for the majority of the time and attacking any intruders that so happen to land on the nest (Theraulaz et al. 1992). The parasite also interacts with subordinate foundresses in a less violent manner, licking their bodies and mounting them, as well as eliciting trophallaxis (the sharing of gut and mouth contents, Theraulaz et al. 1992).

1.5.d Reproduction on parasitised nests

When *P. semenowi* takes over a nest it probably takes over the majority of egg production. The hosts' production of reproductive individuals may halt throughout the time of the parasite's presence on the nest, only recommencing on parasite dispersal or death (Mead 1991). This study concentrated on a single laboratory-held colony, so no firm conclusions can be drawn. Nests in the wild also face a seasonal time constraint so parasite dispersal or death before the end of the nesting season may be extremely rare.

Studies of other *Polistes* social parasites found that host reproduction is similarly affected. In *P. atrimandibularis* attacking *P. biglumis bimaculatus*, host reproduction is decreased, especially the production of reproductives (Cervo et al. 1990). Nest productivity increased compared to similar sized non-parasitised colonies, suggesting either that the parasite 'encourages' an increased work rate from the host or that there is a quicker turnover of parasite brood because of shorter development times. The latter hypothesis is supported in *P. sulcifer*, where parasite brood develops faster than host brood (Cervo et al. 2004). The parasite is perhaps able to have a higher oviposition rate due to not having incurred the cost of building a nest or rearing the first wave of (host) workers.

Studies of *P.* sulcifer attacking *P. dominulus* show that the parasite inhibits ovarian development in workers in a similar way to host alpha dominants, perhaps using similar behavioural dominance to achieve this (Turillazzi et al. 1991).

1.5.e Adoption of Nest Odour

The method by which the parasite persuades host workers and foundresses to accept it on the nest and to care for its own brood is likely to be based on mimicry of host cuticular hydrocarbon "odours", amongst other factors. Studies of *Polistes sulcifer* and *Polistes atrimandibularis* have shown that the cuticular hydrocarbon composition of the parasite changes to resemble that of the host, on takeover of the nest (Bagneres et al. 1996; Turillazzi et al. 2000; Sledge et al. 2001). In both species, as well as *P. semenowi*, the female parasite is observed stroking her

abdomen against the nest surface, perhaps to accelerate adoption of the nest odour and deposit her own compounds (Lorenzi et al. 2004).

Analysis of the parasite's cuticular hydrocarbon signature apparently shows that on takeover there is a severe reduction in the production of unsaturated compounds and a corresponding rise in saturated compounds which resemble those present on the host (Lorenzi et al. 2004). Two possible explanations exist for this observation; the parasite mimics host odour (by biologically synthesising host compounds) or simply camouflages its own odour by covering itself with compounds obtained from the nest or from licking and rubbing host foundresses (Cervo 2006). The parasite itself expresses much lower levels of cuticular hydrocarbon than host wasps, perhaps aiding in the camouflage of its own signature (Lorenzi et al. 2004).

The parasite also seems to add its own unique cuticular hydrocarbons to the nest. Surprisingly, the parasite itself apparently loses from its signature some of the compounds deposited. These species specific compounds might be learnt subsequently by emerging workers, along with the host derived nest odour and used as a template for nest-mate recognition. By adding a parasite specific compound, parasite brood bearing the compound will be recognised as nest mates and not "alien". A schematic of *P. semenowi* takeover of *P. dominulus* nests is shown in Figure 1.5-2.

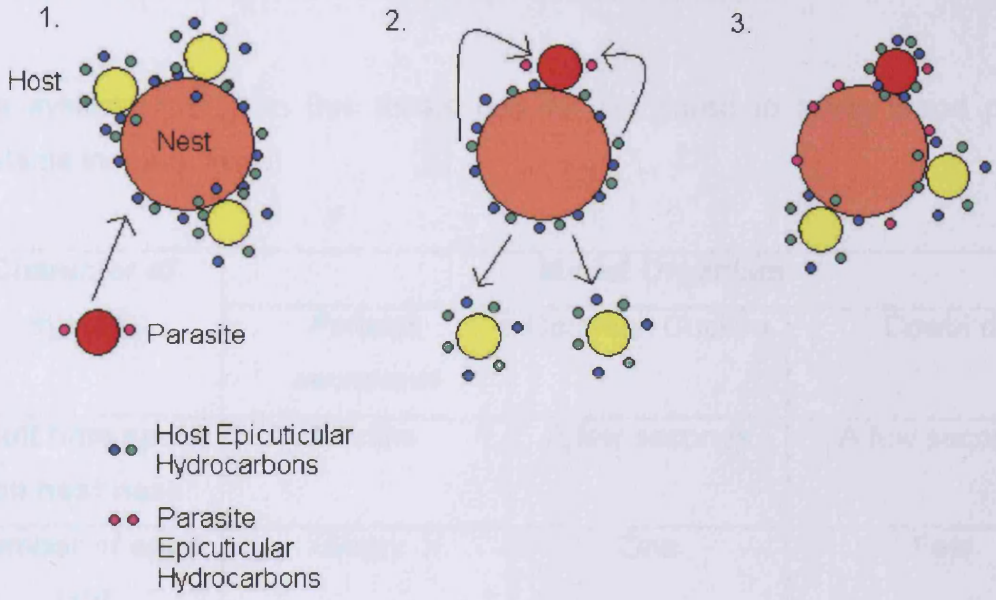

Figure 1.5-2: A schematic representation of *P. semenowi* nest takeover. Step 1 involves physical attack of the host foundresses, driving them off the nest. Step 2 is concerned with the parasite adopting the nest odour. Step 3 heralds the return of host foundresses which now do not attempt to drive the parasite from the nest.

1.5.f *Polistes semenowi* and Avian Brood Parasites

The system studied in this thesis can be compared to avian brood parasite systems in many ways:

Character of system	Model Organism		
	Polistes semenowi	Common Cuckoo	Cowbird
Adult time spent on host nest	Months	A few seconds	A few seconds
Number of eggs laid	Many	One	Few
Mimetic eggs	Unknown	In many cases	No
Host recognition system manipulated so hosts accept hatched parasite offspring	Yes, worker exposure to parasite odour probably leads to acceptance of brood	Yes (mainly via egg mimicry and exaggerated begging)	No
Parasite and Host Offspring Co-habit nest	Yes	No	Yes
Host reaction to parasite adult on nest	Attack of parasite	Attack of parasite	Attack of parasite
Stage of nest in parasite attack	After worker egg laying	After eggs laying	After egg laying
Hatching time of parasite eggs	Faster than host	Faster than host	Faster than host
One parasite female per nest?	Yes	Commonly	Not Necessarily

Table 1-4: A comparison of *P. semenowi* parasitism with the two key types of avian brood parasite systems (Davies and Brooke 1988; Cervo et al. 1990; Cervo and Dani 1994; Kilner et al. 1999; Davies 2000).

In both cases, the parasites exploit a "rule" in order to have their brood accepted by the host. In the case of avian systems, an egg is accepted only if the host can be sure it laid it (so host brood must already be present in the nest when the parasite attacks) and in species where egg mimicry occurs must also appear phenotypically like a host egg. In *Polistes*, the parasite must exploit the rule that "if a wasp or brood bears the cuticular signature of the nest, it is a nest-mate".

1.6 Host Response to Parasitism

Foundresses on *P. dominulus* nests faced with *P. semenowi* attack have several options potentially open to them:

- Stay for possible reproductive concessions. Studies of *P. atrimadibularis* (Cervo et al. 1990), *P. sulcifer* (Dapporto et al. 2004) and *P. semenowi* (Mead 1991) suggest hosts may still get reproductive benefits, either during parasitism or through parasite abandonment.
- Stay and fight the parasite reproductively through egg-laying (Dapporto et al. 2004), differential provisioning of related brood versus parasite brood and differential oophagy.
- Abandon in order to re-nest or join other nests as a helper or usurper (Makino 1989).

The parasite may need to encourage the host to stay and help, as this is the only way it can have its own brood reared.

1.7 Aims of this thesis

In this thesis I hope to investigate whether *P. dominulus* has developed counter adaptations against *P. semenowi*, as well as whether the parasite in turn has its own counter adaptations to keep hosts on nests:

1. Chapter 3 is concerned with abandonment by *P. dominulus* nests in response to *P. semenowi* attack.
2. Chapter 4 looks at aggression and effort upon parasitised nests. Is parasitism costly in terms of productivity or does the parasite exploit the hosts by increasing their work rate compared to unparasitised nests?
3. Chapter 5 ascertains whether existing microsatellite markers for *P. dominulus* also amplify in *P. semenowi*.
4. Chapter 5 investigates the possibility that *P. dominulus* can directly reproduce on nests parasitised by *P. semenowi*.
5. Chapter 5 elucidates whether the host foundresses have developed ways of fighting back against the parasite through brood destruction or provisioning.

Chapter 2. **General Methods**

2.1 Introduction

The research in this thesis is based upon one year of laboratory populations (2003) and two years using field populations (2004-2005). Samples for use in molecular studies in the laboratory were taken from the field in 2004 and 2005.

2.2 Field Studies

The populations of *Polistes dominulus* and its parasite *Polistes semenowi* used in this study were within a 10km radius of Conil de la Frontera (latitude 36.2764N, < 10m altitude), a coastal town situated approximately 40km SE from the major port of Cadiz, in South Western Andalucia, Spain (Figure 2.2). The majority of field work was performed by Edward Almond between February-June 2004 and 2005, with minor assistance from Lorenzo Zanette. This work consisted of behavioural observation, population monitoring, video recording and sample collection. The *P. dominulus* populations, and *P. semenowi* activity in this area have been studied previously but this study represents the first major observational work on *P. semenowi* (Cant and Field 2001; Shreeves et al. 2003). Samples for the 2003 laboratory study were also taken from these sites.

2.2.a Study areas

The population studied occurred within agricultural land surrounding Conil de la Fronterra, in Andalusia, Spain (Figure 2.2). Weather data for the area are listed in Appendix 1, the conditions producing a colony cycle in *P. dominulus* similar to other temperate zone species (Reeve 1991).

The farmers in the surrounding countryside use the cactus *Opuntia ficus-indica* L. (commonly known in Britain as the Barbary-fig or prickly pear cactus), in order to demarcate their fields and control the movements of farm animals. This cactus provides good nest sites for *P. dominulus*, probably because the spines deter vertebrate predators (personal observation). The cactus allows elevated nest positions (up to 3m high), perhaps lessening exposure to ground predators such as ants, which, if allowed, will rob the nest of its entire brood (personal observation, n=12 nests). It also shields nests from coastal winds, which are renowned in the area. The surrounding farm area provides a source of nectar, prey and wood pulp for nest construction from wild flowers in pastoral land, and irrigation for crops also gives the wasps a ready supply of water.

The cactus (Figure 2.1), whilst sometimes painful to work with, provides easy access to the *P. dominulus* nests. The nests themselves are open-celled and cell contents are easily ascertained with the naked eye. This ease of access to nests combined with their open structure allowed careful observation and manipulation of both adults and brood when necessary in the study.

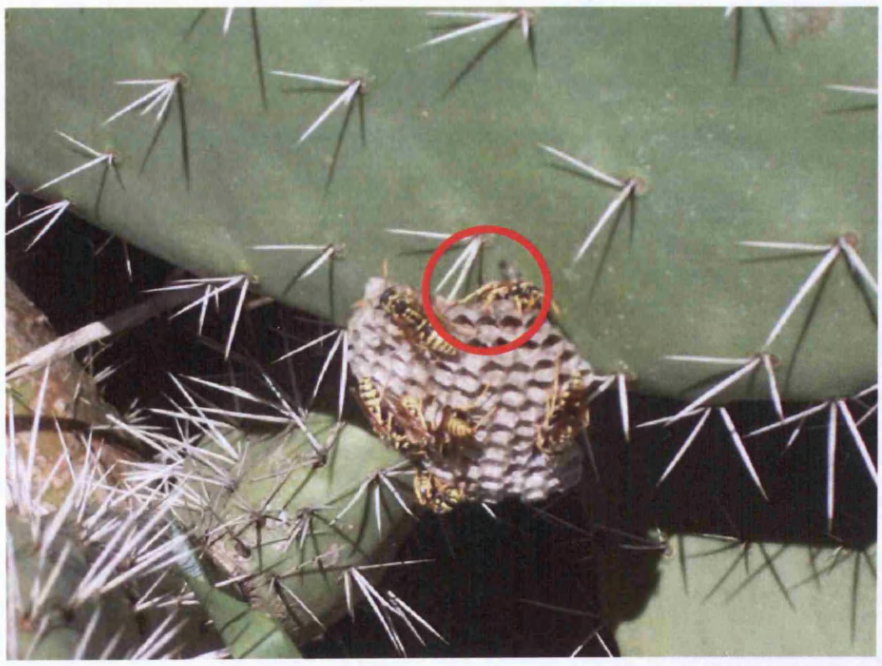

Figure 2.1: *Polistes dominulus* nest with the parasite *Polistes semenowi* (ringed) on *Opuntia ficus-indica* L.

In the 2004 field studies, two sites were used; Site CF ("Farm") was a large area of cactus surrounding an area of grazing land for cattle, it in turn surrounded by arable land. Site G ("Gate") consisted of two linear tracts of cactus, again surrounded by arable land, but with a lightly used farm track adjacent to it. In both cases, the majority of nests were found to occur in areas exposed to the sun rather than on shady sides.

In 2005, Site G had unfortunately suffered a catastrophic building works, destroying the majority of cactus, so only Site CF was used for the majority of experiments. Another site, Site P (a single 100m row of cactus separating two fields of crops), was used for a small (n=6 parasitised nests) study investigating brood removal in parasitized nests.

The surrounding farm areas of Conil contained many other sites with *P. dominulus* activity that were not used in this study in 2004-2005 but were used as a source of *P. semenowi* in 2003.

Name of site	Years used	Experiments performed	Monitoring period	Location
CF	2003 2004 & 2005	Sample collection Abandonment survey, Video analysis of parasitized nests	February-June, collections in June.	3km along CA-2144 in pastoral land.
G	2003, 2004	Abandonment survey, Video analysis of parasitized nests, Sample collection	February-June, collections in June	5km along CA-2144, row of cactus along farm access route
P	2005	Brood removal experiment	April, no collection	CA-213/N-340 intersection.

Table 2-1: Assigned names of sites used in the study, along with information on how they were used.

Figure 2.2: Red arrow indicates area of study sites

2.2.b Initial Site Preparation

At first, the position of *P. dominulus* nests in each site had to be marked and recorded. Pieces of electrical tape were skewered upon the cactus spines near to nests, to provide a visual marker. Waterproof ink markers were used to label the cactus near each nest with a unique identification number. Nests were labelled only after 3-4 cells had been built, to avoid causing the proto-nest to be abandoned. This study-wide census for new nests was continued throughout the period of the investigation, for purposes which will be made clear later, twice a week.

2.2.c Marking individual adults

In order to distinguish adult resident *P. dominulus* foundresses from newly joining individuals, newly hatched "workers" and, in the case of parasitized nests, the parasite *P. semenowi*, individuals on nests to be studied were individually marked. Four dots of enamel paint were applied to the thorax, as shown in Figure 2.3. There were a total of 7 colours used in marking, giving 840 possible permutations, more than sufficient for the experiment's needs.

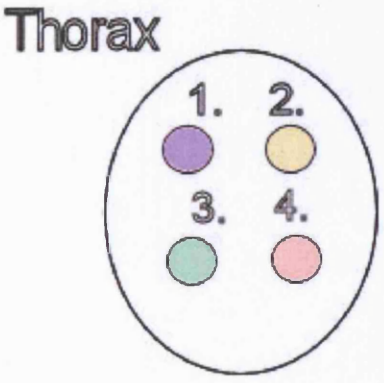

Figure 2.3: Marks made on the thorax of adult wasps in order to distinguish between individuals on a nest. The sequence of the four coloured dots was unique to the individual that bore them.

Previous studies of the *P. dominulus* populations used here indicated the vast majority of foundresses returned to the nest at night (Shreeves et al. 2003). Wasps were therefore captured for marking between the hours of 10.00p.m.-6.00a.m., normally several hours after sunset when temperatures reached their lowest. At these times the wasps are also relatively docile and easy to capture and manipulate. Forceps were used to grab wasps and place them in a sealable plastic zip-lock bag, held under the nest to capture any wasps attempting to flee by dropping off the nest. Wasps were kept in a dark, cool-bag after collection until marking occurred.

Wasps were marked using the head of an entomological pin, the wasp itself being held in gloved hands, taking care not to injure it. Marking took around 1 minute and the wasps were usually returned to their respective nests within an hour of being collected. A minority were kept in a refrigerator for several hours before returning them if bad weather conditions prevented safe return immediately.

Each wasp was released within 2 metres of its nest. After marking, wasps were not included in studies for at least 24 hours to allow them to return to their normal behaviour. The paint was reasonably durable, but on occasion spots partially or fully rubbed off. Any wasp seen to have lost any of its marking was identified by checking for absentees from the nest in the most recent census (for census methods see section 2.2.d). Wasps were then re-marked in the next round of wasp marking.

2.2.d Nest Censusing

Censusing of nests is defined as the recording of individuals present on a nest at the time of observing. Censusing took place for several distinct reasons;

1 Determining the number of foundresses resident on a nest.
2 Recording nest abandonment.
3 Discovering usurped nests (either usurped by conspecifics or the parasite *P. semenowi*).
4 Determining the identity of the dominant individual on the nest.
5 Monitoring the number, position and demography of brood within the nest.

Foraging in *P. dominulus* occurs during the daytime, so any estimate of foundress numbers would be inaccurate due to absent foraging wasps. The night time, however, allows examination of nests without having to interfere too heavily with them, thus minimising the risk of influencing wasp behaviour or damaging nests. Therefore, two temporal forms of nest censusing were used; **day censusing** and **night censusing**.

2.2.e Night censusing

Night censuses were performed twice a week. As stated previously, wasp activity at night is negligible; indeed the only activity recorded was nest defence or abandonment in response to raids by ants. A series of censuses at night can therefore be reasonably sure of recording both the true number of wasps resident on a nest as well as serving to identify these wasps if they are marked (Shreeves et al. 2003). During the preparation phase, night censuses were performed in this way to identify any unmarked individuals on a nest, to ensure all foundresses were marked prior to worker emergence.

This methodology has the advantage of recording all potential egg layers prior to collection, so that when molecular techniques are used to assign maternity, one can be reasonably certain of the results obtained.

A L.E.D. head mounted torch was used to illuminate the nest area at night. Previously marked wasps and unmarked individuals were recorded. Unmarked wasps could, through examination of the nest, be assigned to a hatched pupal cell or identified as an "alien" wasp that had joined the nest. In both cases the wasp would be marked during the next marking cycle.

The absence of *any* host wasps on a nest for 3 consecutive night censuses (and all day censuses between them) was taken as a sign of nest abandonment, as this represents over a week of observed absence. Any possible causes of abandonment were also recorded. These include damaged or destroyed nests, absent nests with nest residents gathered around the nest site, ant raiding parties and total brood destruction.

2.2.f Day censusing

Day censusing was performed in three specific ways:

1. Abandonment censusing
2. Brood mapping and censusing
3. Dominance determination

2.2.f.i. Abandonment censusing

Abandonment censusing was perhaps the simplest and least invasive technique, and was performed 1-2 times per day after initial site preparation (see section 2.2.b). The wasps on nests used to study abandonment were not individually marked after

marking the nest position. This minimized stress to the wasps, which could have caused abandonment over and above the natural baseline rate.

The numbers of wasps present upon the nest, as well as any unusual occurrences such as presence of ants or water damage due to rain, were noted in the census. If a *P. semenowi* adult was spotted close to (within 2m), or upon, the nest then this too was noted. If host wasps abandoned the nest after attack by a parasite, and did not return to the nest over the next three abandonment censuses, then they were deemed to have abandoned the nest due to parasite attack. Likewise, if their absence occurred for three censuses or more and another, non-parasite related factor (such as weather damage), was observed, then the nest was deemed to have abandoned due to other causes.

2.2.f.ii. Brood mapping and censusing

Brood mapping and censusing was easiest performed pre-sunset, when there was still some light available but wasp activity was reduced. Nests consist of a pattern of hexagonal cells that tessellate perfectly and so can easily be mapped using hexagonal graph paper (Figure 2.4). The open celled structure of the nests, combined with the availability of tiny Microlite torches that can be focussed into the cells, meant that brood surveying was relatively simple and accurate. By making these maps initially, the contents of every cell could then be recorded consistently over the subsequent censuses and the development of any brood in these cells monitored.

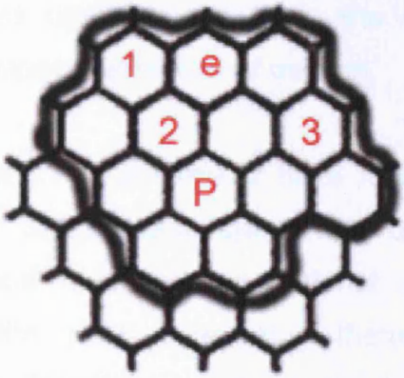

Figure 2.4: A typical brood map. "e" indicates egg, "1" small larvae, "2" medium larvae, "3" large larvae and "P" pupae. The outline indicates the nest outer edge.

Maps were not always necessary when recording brood information. In the abandonment experiment, only the number of the different stages of brood (Figure 2.4) were recorded, not their position. This brood surveying took place twice a week for nests involved in the abandonment experiment, and once per week in the other experiments (supplementing the weekly brood mapping).

Where position of brood was useful to record, such as with the experiments involving the host feeding of brood, nests were censused every week. This was regular enough to allow the development of brood to be monitored between larval stages and also allow any brood disappearance to be determined unambiguously. If a stage 1 larva disappeared and an egg laid in its place, it would take more than a week for the egg to hatch and grow into a stage one larva. Therefore, mappings made less regularly would perhaps miss this destruction.

2.2.f.iii. Dominance Determination

The role of dominant individual on a nest is defined in Chapter 1. In the experiments involving parasite takeover of a nest, with subsequent parasite removal, a matched control nest had to be found. This nest was an unparasitised host nest with approximately the same number of host foundresses, number of cells and composition of brood. The parasite occupies the role of dominant foundress on

parasitized nests, so it was useful to determine the dominant foundress on the control nests in order to compare behaviour of the two.

Dominant individuals in *P. dominulus* have markedly different behavioural patterns than subordinates. Subordinates spend a lot of time off the nest, foraging for food, water, nectar and nest building material (Gamboa 1978). Dominant individuals rarely leave the nest, concerning themselves mainly with social interactions and brood care. Dominant individuals meet returning foragers and often induce the forager to regurgitate liquids or release solid foods held in their mouths to the dominant (Theraulaz et al. 1991). Table 2-2 lists the main behavioural differences between dominant and subordinate individuals.

Behaviour of dominant individual	**Behaviour of subordinate individual**
Raises antennae, waggles antennae, and strokes antennae over other wasp in interaction.	Lowers antennae and remains immobile when interacting with dominant wasp.
Remains on nest, rarely foraging for food.	During foraging times, rarely on the nest, partakes in foraging for food and wood pulp.
Exchange of solid food and liquids are generally directed towards the dominant from subordinates (Westeberhard 1969; Marino Piccioli and Pardi 1980).	Targeted for trophallaxis, often actively seeks tropholaxis with dominant.

Table 2-2: Behaviours of subordinates vs. dominants based on previous studies and personal observation.

Cant and Field (2001) determined dominance using daytime censuses, recording presence or absence from the nest of marked wasps. The dominant was identified by it being seen on the nest for at least three occasions more than any other wasp, after at least 20-30 censuses. This method was followed in my study, performing half-hourly censuses between the hours of 10 a.m. to 5 p.m. on calm, sunny days.

To augment this approach, half hour behavioural observations of these nests were carried out during this census period, specifically focussing on dominance interactions between wasps on the nest. This latter approach has been used in isolation to determine dominants in previous studies of *P. dominulus* (Dapporto et al. 2004). A combination of the two approaches left little ambiguity as to the identity of the dominant.

2.2.g Video Recording

Because *P. dominulus* nests often consist of over a hundred cells and there are often several individuals on the nest, observing and recording feeding events for every cell via field observation is impossible. By obtaining high quality video recordings of a mapped nest with marked individuals, feeding and other interactions could be scrutinised at a later date. The camera allows lens magnification of images captured and footage can be viewed frame by frame in the laboratory.

2.2.h Nest Collection

Nests and wasps involved in the filmed experiments were collected in order to be able to determine parentage of the brood in the laboratory. Nests and their respective wasps were collected during the night immediately after filming, to minimise risk of the cell contents changing or the nest being lost though predation or abandonment. A brief nest census was performed to ensure all marked individuals were present to be collected. If any individuals were missing, nests were left for 24 hours and another census made. If the wasp was still not present, the nest was collected and further night censuses made in the vicinity to try locate the absent individual. Day censuses, performed as parts of the experimental routine, were also used to locate and capture absent wasps.

Nests were collected by torchlight. A receptacle was first placed under the nest to catch falling wasps, then forceps were used to prise the nest by the pedicel from the cactus and the nest was placed in a Ziploc bag. If done with care, adult

wasps remained motionless on the nest during this process, meaning both nest and wasps could be obtained swiftly and with minimum damage to either.

Nests and their respective wasps were stored in a +4 degrees refrigerator until they were taken to the University Of Cadiz where -80 degrees Celsius storage was available. In preparation for -80 degrees Celsius storage, nests were mapped and labelled and wasps placed in individual tubes. Samples were usually taken from the field to the -80 degrees Celsius storage within four days. Samples were transported back to the U.K. either as live specimens or on dry ice.

2.3 Laboratory Studies

Two methodologically different activities were performed in the U.K.; **Microsatellite analysis** of the adult wasps and their brood, and **Video Analysis** of the behaviour of adult wasps.

2.3.a Microsatellite Analysis

In order to obtain genotypes, the samples underwent the following steps:

- **Sample selection**
- **Sample preparation.**
- **DNA extraction and purification.**
- **PCR amplification of specific microsatellite loci.**
- **Determination of PCR product sizes.**

Methods for each stage are described below, with detailed protocols given in Appendix 3:

2.3.b Sample selection

All adult wasps from each nest and between 12-36 host brood were selected for microsatellite analysis from 17 parasitised nests (see Chapter 5). Nests with below 12 brood had all brood analysed while brood from larger nests were chosen at random.

2.3.c Sample preparation

Tissue from either adult wasps (using half a thorax) or brood had first to be prepared for DNA extraction. The protocol used was based on protocols used previously in other laboratories with *P. dominulus* (Strassmann 1996) as well as in the University College London laboratory with *P. dominulus* and *Liostenogaster flavolineata* (Bridge 2005).

A grinding buffer was used to homogenise the sample prior to DNA extraction. SDS in the buffer was used to lyse cell membranes to release nucleic acids for extraction, EDTA was used as a chelating agent of Mg which otherwise may have promoted nuclease activity and thus degraded the DNA.

2.3.d DNA Extraction and Purification

An Ethanol/Acetate extraction method (Strassmann 1996) was used to extract genomic DNA from the homogenised tissue. The acetate served to remove the salts and SDS from the extraction solution present in the grinding buffer. To extract pure genomic DNA from the solution, ethanol was used.

The purified DNA was dehydrated then diluted with ddH_2O. 2µl of this extracted DNA solution was then run on a 1% agarose gel containing ethidium bromide, in a TBE buffer, to determine the success of the extraction.

2.3.e Locus Characterisation: Primer Development and Optimisation

Although microsatellites to be used have already been shown to work well in *P. dominulus*, the use of fluorescent primers as opposed to radioactive labeling in the previous study (Cant et al. 2006) meant that primer sequences were first analysed *in silica.* The published primer sequences were evaluated (Jellyfish software http://jellyfish.labvelocity.com) to ensure that they met the following requirements;

- Primers used had to be a minimum distance from the repeat motif to avoid excessive "stutter" production in PCR.
- Primers had to be of sufficient size and complexity (defined as the G-C content) to ensure stringent annealing at only the single site.
- The 3' end of primer had to have a higher A-T content to ensure complete annealing prior to the Polymerase mediated extension phase of PCR.
- The microsatellite region to be amplified should have ideally been in the range of 100-250 b.p. to allow accurate, unambiguous scoring of products. Too low a size risks confusion with primer-dimers and other side products of PCR. Too large a size means products are generally less easy to score accurately. See Figure 2:5 for product size ranges.

2.3.f Using primers designed for *P. dominulus* with *P. semenowi*

Studies in birds have indicated that the likelihood of a taxon amplifying at a particular locus is negatively correlated with the phylogenetic distance from the species the locus was cloned in (Primmer et al. 1996). As *P. dominulus* and *P. semenowi* are closely related (Carpenter et al. 1993), existing loci that amplify in *P. dominulus* (Henshaw 2000) are likely to amplify in the parasite species. This study is the first to use microsatellite analysis on the parasite and confirms that the loci used amplify and are variable in *P. semenowi.*

2.3.g PCR amplification of specific microsatellite loci

A detailed protocol is given in the appendix. In order to increase sample throughput, multiplex PCR was used. This simultaneous amplification of five microsatellite loci in a single reaction vessel required prior knowledge of allelic size ranges (Cant et al. 2006) for each of the loci, in order to avoid products of one locus overlapping with another labeled with the same fluorescent dye (Figure 2:5). Thermoprime Plus Taq DNA Polymerase was used in replicating the DNA template in the PCR reaction. The Mg^{2+} concentration and annealing temperature of this multiplex reaction was optimised using temperature and concentration gradients.

2.3.h Sizing of amplified PCR products

PCR products were separated using an ABI 3100 capillary sequencer using dye set C. 10µl of a mix of formamide and Genescan 500 Rox size standard (a ladder of ROX fluorescent labelled DNA fragments of known size) were added to wells in a 96 well plate, in a 90:1 ratio. 1.1µl PCR products was added to the wells and the plate heated for 5 minutes at 95°C then immediately placed on ice. The formamide and heating both serve to separate the DNA strands prior to sequencing. The ROX size standard allowed each sample run to be automatically sized by the sequencer and because the same size standard was used for every sample, sample sizes would be sized according to the same criteria in each sequencing event.

The ABI 3100 is an automated sequencer and once samples were loaded and plate files imported into the computer interface, no further user action was required. Results were exported as sample files which were examined in Genescan software (ABI).

2.3.i PCR product scoring

PCR products from the five loci used were distinct in either their expected size range (Cant et al. 2006) or the colour of their fluorescent label, to allow unambiguous scoring of alleles (see Figure 2:5). Artefacts such as primer-dimer complexes or degraded DNA-primer complexes generally fell within the 0-100 b.p. range, whereas alleles were found above 120 b.p., therefore minimising misidentification of these artefacts as alleles.

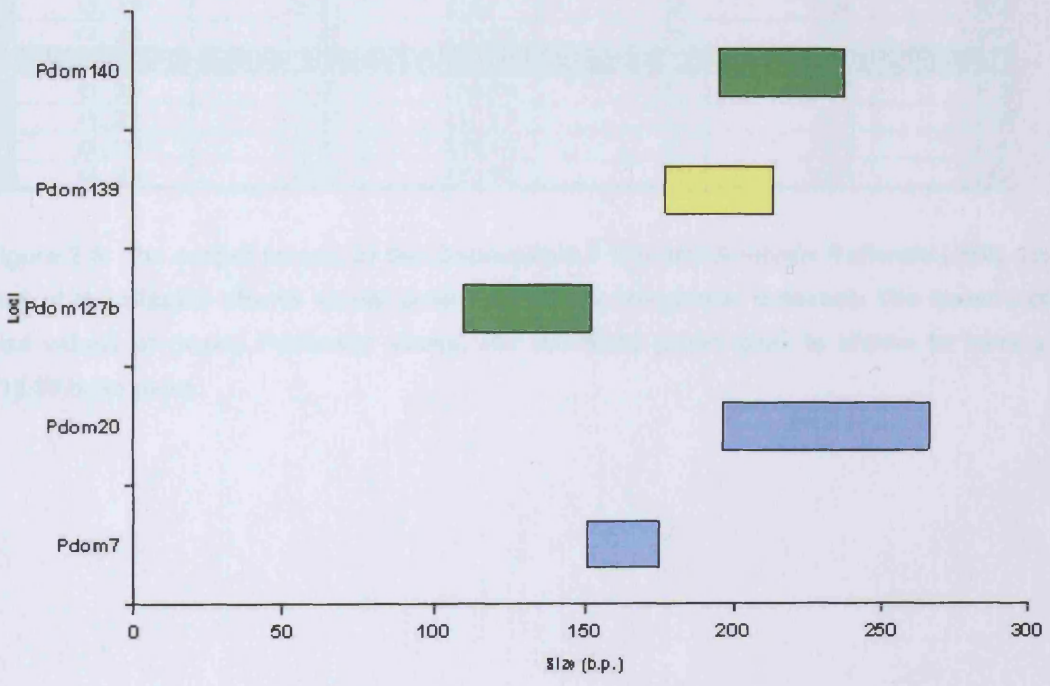

Figure 2:5: Allele ranges of the 5 microsatellite loci used based on work performed in the Field laboratory. Colours of bars indicate the dye colour used (NED, FAM or HEX).

Each of the loci produced a distinctive band of size peaks when viewed in Genescan (Figure 2:6), making identification of alleles associated with loci relatively easy. As alleles for all loci were multiples of three base pairs apart, scoring was done by rounding the absolute peak score to the nearest expected allele value. Where alleles were intermediate between two expected values, samples were rerun and rescored. Samples were re-run in most cases to check for consistency in sizing.

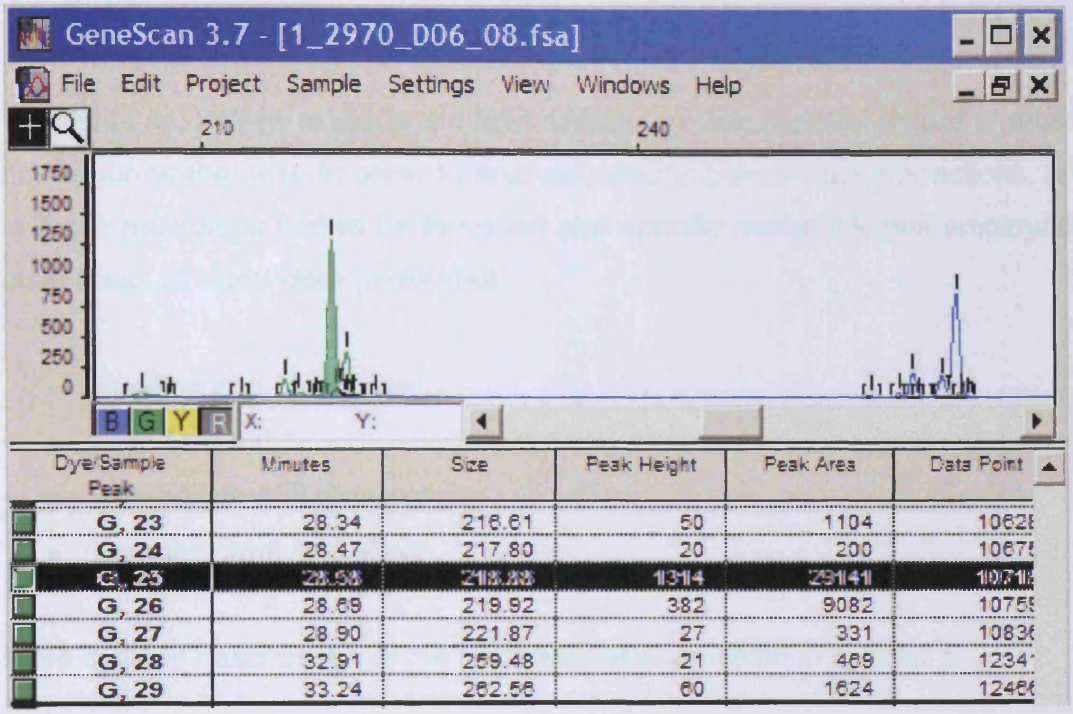

Figure 2:6: The output format of the Genescan 3.7 Genetic Analysis Software (ABI). The upper part of the display shows visual peak data of the fragments detected. The lower table gives size values of peaks. From the above, the left-hand green peak is shown to have a size of 218.88 base pairs.

2.4 Video Analysis

The video recordings made in the field season provide a useful record of adult wasp behaviour on the nest. In order to analyse specific behavioural interactions, analysis of these recordings had to be focussed and specific methodologies employed. Four main areas of study were performed:

- Feeding and brood care
- Nest building
- Aggression and dominance
- Foraging time and effort

More detailed descriptions of the methods used are given in chapter 5.

2.5 General Statistics

Data quoted are as estimates ± standard error unless otherwise stated.

Chapter 3. **Abandonment**

3.1 Introduction

In this chapter I test the hypothesis that *P. dominulus* abandons its nest in response to attack by the parasite *P. semenowi.* Usurpation of the nest by *P. semenowi* represents a potential catastrophe for the host. The unrelated parasite aims to take over brood production; producing offspring that are completely unrelated to the host foundresses (see Chapter 5). Hosts that succumb to parasitism seemingly lose both direct and indirect fitness benefits of staying on the nest as they help raise only non-kin. The only benefit in staying is to rear the remaining host brood and attempt to directly reproduce, or to inherit the nest should the parasite leave (see Chapter 5, Mead 1991). This benefit could be potentially large, depending on the number of host larvae in the nest at the time of takeover, and whether the hosts can manipulate the caste of such larvae so they become reproductives rather than sterile workers. Even so, being parasitised represents a huge fitness cost in terms of loss of future reproduction on the nest.

In investigating abandonment as a response to parasitism, other reasons for abandonment must also be taken into account. A *P. dominulus* colony can abandon for many reasons, which can be grouped into three broad categories; stochastic factors, predation and *P. semenowi* parasitism.

3.1.a Stochastic Factors:

These factors are concerned with interactions not directed specifically at nests. In the areas studied, these include weather conditions (the area is renowned for high wind speeds, see appendix for weather data) and disturbance through agriculture. They can be seasonal (i.e. weather) or random (i.e. cattle damage) in their action (Yamane 1996).

3.1.b Predation:

P. dominulus nests represent a concentrated, immobile source of protein for would-be attackers, predominantly birds and ants in my study populations.

In other *Polistes* species, such as *Polistes chinensis* it is estimated up to 8.6% of the population of pre-emergence colonies are destroyed by two species of ant, *Lasius niger* and *Pristomyrmex pungens,* with a further 2.2% of post-emergence nests destroyed as well (Miyano 1980). The development of single petioles to connect nests to substrates, as well as deposition of ant-repellent compounds are evidence of the evolutionary pressure such predation has exerted (Jeanne 1975). I observed only low levels of abandonment associated with ant invasion of nests (n=4; 2.3% total nests 2004, n=4; 3.2% total nests 2005). It is likely that some of these instances were due to ant opportunism after periods of heavy rainfall and wind, where ants raided nests which were already abandoned.

The adult wasps, as well as nests, are target for attack by birds (e.g. by the European Bee-Eater *Merops apiaster*, Fraser Simpson, personal communication). Nests attacked in this manner are usually displaced or heavily damaged due to the bird tearing up the nest structure to obtain the larvae, so can easily be identified and differentiated from nests abandoned due to other factors.

3.1.c Foundress Mortality

Nests could seem to be abandoned due to the deaths of each of the foundresses present; for example through predation by arachnids, which spin their webs on the cactus, or through avian and reptilian predators present at the field site. Disease or poisoning from agricultural pesticides could also play a part. If this was the case, then one might expect larger groups to survive longer than smaller groups.

One insurance based theory about the origins of eusociality centres on differential survival. "Survivorship Insurance" (SI) predicts that larger groups, by

having more members, are better protected against total group failure through the individual deaths of all foundresses on the nest (Nonacs 1991; Reeve 1991; Queller 1994; Nonacs and Reeve 1995). Simply put, the more foundresses there are on a nest, the greater the chance there is of a foundress being present to care for brood should another foundress die. However, in my population of *P. dominulus*, SI has not been found to operate (Shreeves *et al.* 2003).

3.1.d Parasitism:

Foundresses on *P. dominulus* nests faced with *P. semenowi* attack have several options open to them:

- Stay for possible reproductive concessions or to inherit the nest. Studies of *P. atrimadibularis* (Cervo *et al.* 1990), *P. sulcifer* (Dapporto *et al.* 2004) and *P. semenowi* (Mead 1991) suggest hosts may still get reproductive benefits, either during parasitism or through parasite abandonment.
- Stay and fight the parasite reproductively through differential provisioning of related brood versus parasite brood or differential brood destruction (Dapporto *et al.* 2004).
- Abandon in order to re-nest or to join or usurp other nests (Makino 1989).

Chapter 5 investigates whether hosts do either directly reproduce or differentially care for existing offspring on parasitised nests. This chapter is concerned solely with the latter option, abandonment.

3.1.e Abandonment of nests in *Polistes* in response to social parasites

Whilst absence of some foundresses post-attack has been noted in several studies (Mead 1991; Lorenzi *et al.* 1992), the complete abandonment of nests by foundresses in response to brood parasite attack has yet to be recognised in *Polistes*. Abandonment by *Polistes* workers after conspecific usurpation has been recorded, but workers usually hatch out *after* the nest has been usurped and so do not abandon as a direct response to the usurpation event, but rather to cues that the nest is being parasitised (Klahn 1988; Makino 1989; Makino and Sayama 1991).

3.1.f Abandonment in hosts of avian brood parasites

Nest abandonment has been studied among hosts of avian brood parasite (Rothstein 1976; Graham 1988; Hill and Sealy 1994; Rutila *et al.* 2002). Abandonment of nests that have been brood parasitised has been noted in Reed Warblers (Davies *et al.* 1996), Red Bishops (Lawes and Kirkman 1996), Superb Fairy Wrens (Langmore *et al.* 2003), Clay Coloured Sparrows (Hill and Sealy 1994) and many hosts of cowbirds (Hosoi and Rothstein 2000; Langmore *et al.* 2003).

Some authors noted that abandonment was not in response to parasitism *per se*, but to factors related, yet not exclusive to, parasite attack such as egg damage (Hill and Sealy 1994). Avian brood parasites attempt to place their eggs in host nests without being discovered by the host, relying on stealth (avoiding host) and deception (mimetic eggs) to get their egg accepted (Dawkins and Krebs 1979). One study, on parasitism by Horsfield bronze-cuckoos of superb fairy wrens, has shown that hosts abandon parasite young rather than just their eggs (Langmore *et al.* 2003). This is presumed to have occurred due to a combination of accurate egg mimicry by the parasite combined with high rates of parasitism, selecting for alternative means of host defence.

Polistes brood parasite systems differ from Cowbird and Cuckoo systems in a key way: hosts in *P. dominulus* are **always** subject to and respond to parasite encounter when the parasite initially attacks the nest (Cervo *et al.* 1990; Mead 1991;

Cervo and Dani 1996). The attack is usually violent and the parasite remains on the nest after attack. The parasite apparently camouflages itself with the host epicuticular hydrocarbon signature (Turillazzi *et al.* 2000; Dapporto *et al.* 2004; Lorenzi *et al.* 2004). Egg destruction by the parasite, should it occur, also happens **after** the actual physical takeover (Cervo and Lorenzi 1996), so if this causes abandonment rather than parasite attack *per se* then this should be apparent from the timing of abandonment.

Evolution of responses to parasite presence on the nest has occurred in birds and might be expected in *P. dominulus.* In studies of Reed warbler responses to cues of parasite presence, hosts presented with a stuffed cuckoo at the nest showed significantly increased rates of egg rejection (Davies *et al.* 1996). Hosts that did not have visual contact with the cuckoo rejected eggs at the baseline rate predicted by local parasitism rates.

P. dominulus always receives an obvious and unambiguous cue that it is being parasitised. An anti-parasitism measure such as nest abandonment could evolve as a response to this cue.

3.1.g Nest Choice by Parasites

P. semenowi should be expected to parasitise nests that will enable it to maximise its reproduction. The parasite might choose nests with a large number of cells to lay in, large numbers of large host brood to provide many workers and several foundresses to aid the parasite during the initial phase of parasitism. A previous study of the populations used in this thesis has shown that *P. semenowi* preferentially attacks nests with larger group sizes (Shreeves *et al.* 2003). Such parasitised nests were also less likely to be abandoned (Shreeves *et al.* 2003). Host foundresses on such nests are faced with the choice between abandoning a large number of related brood which could die as a result, and staying in order to rear them. The benefits of staying, therefore, might be higher in large nests, if the number of larvae per foundress is greater on larger nests. Parasite selection of large nests could therefore be doubly advantageous, affording a large workforce and capacity for parasite brood, with foundresses that are more likely to stay and help.

3.1.h Options for abandoning hosts

Abandoning *Polistes* hosts have several options open to them;

1. Join other nests as subordinates
2. Takeover other nests
3. Overwinter and breed the following season
4. Build a new nest

The ease of joining another nest is very much dependent on time in the season. Prior to major brood production, groups are relatively dynamic with new members joining and foundresses leaving (Nonacs and Reeve 1995; Shreeves *et al.* 2003). At the time of *P. semenowi* attack, many nests contain several mature larvae. At this time, groups become more aggressive towards newcomers as the costs of accepting an undesirable individual, who may usurp the nest and destroy brood, increase (Reeve 1989; D'Ettorre *et al.* 2004). Foundress aggression in general increases as the season progresses (Cant *et al.* 2006).

Potential *P. dominulus* usurpers attacking at the time of parasite attack also face this increased aggression, which might increase the risk of injury or mortality. Also, the parasite attacks in the period just prior to worker emergence (see Chapter 1). Any abandoning wasp aiming to usurp a nest might have to fight both foundresses and newly hatched workers, making likelihood of success smaller. Some nests may have been naturally abandoned through foundress mortality and therefore easy to take over. However, it has been shown in *P. dominulus* that some foundresses do not initiate their own nests, but simply "sit and wait" for such nests to become available (Starks 1998). These "sit and wait" individuals have not had to expend energy building a nest or foraging, so would be expected to be able to out-compete abandoning foundresses for access to the vacant nests.

Leaving the nest and reproducing the next season could potentially be an alternative reproductive strategy after nest abandonment. Certainly, it has been observed in *P. fuscatus* that the first workers sometimes disperse and breed the next season (Reeve *et al.* 1998). However, to do this would require the foundress to

survive another hibernation period with internal energy stores reduced due to its earlier nesting activities.

Building a new nest may be a viable option, but time constraints due to resource limitation might impose a large cost in such an action. A new nest would have little time left in the season to produce offspring before the onset of winter. Given the high levels of nest abandonment observed in this population, however, re-nesting may still occur (Makino 1989; Kumano and Kasuya 2006).

3.2 Aims

The main aims of this chapter are to investigate:

1. the pattern of parasite attack in the season.
2. whether parasites choose nests with certain characteristics which may influence host non-abandonment.
3. whether *P. dominulus* foundresses abandon in response to being parasitised by *P. semenowi*.
4. whether *P. dominulus* foundresses abandon nests in response to the initial parasite attack.
5. other factors involved in survival of *P. dominulus* nests.

Aims 3 and 4 can be differentiated by analysing abandonment in two specific contexts;

1. Aim 3 by examining whether parasitised nests abandon at a greater rate overall than unparasitised nests. This might imply that hosts stay on nests that are parasitised initially and rear any remaining brood, then leave.
2. Aim 4 by focusing on abandonment within 1 census of the parasite's initial attack. Hosts in this case do not stay on the nest, they leave at the point of the initial attack.

3.3 Methods

3.3.a Initial Site Preparation

In 2004, farm and gate sites were used (n=196 nests) and in 2005 solely the farm site (n=130 nests). An initial census recorded and labelled the position of *P. dominulus* nests using electrical tape. Nests were labelled only after 3-4 cells had been built, to avoid causing the proto-nest to be abandoned. These site-wide censuses for new nests were continued twice a week throughout the study period until the abandonment survey was initiated.

The wasps on nests used to study abandonment were not individually marked. This minimized stress to the wasps, which could have caused abandonment over and above the natural baseline rate.

3.3.b Recording abandonment

"Abandonment censuses" were taken daily, after 3pm, recording parasite attack and the number of foundresses present on the nest, along with any other relevant observations (such as attack by nest predators). Parasite presence was defined as a *P. semenowi* adult being within 2m of, or upon, the nest. A weekly brood census was carried out to record nest size and brood number and composition (see Chapter 2).

I define abandonment due to parasite attack as follows: if host wasps did not return to the nest during the three abandonment censuses following recorded parasite presence, then they were deemed to have abandoned the nest due to parasite attack. Likewise, if they were absent for three censuses or more and another, non-parasite factor (such as weather damage) was observed, then the nest was deemed to have abandoned due to other causes.

3.3.c Are parasites selective in their choice of host nests?

Rather than being a response to parasitism, differences in survival rates could occur between parasitised versus non-parasitised nests because *P. semenowi* chooses nests that are less likely to fail than average (Shreeves *et al.* 2003). This may also mean that nests attacked by the parasite are less likely to abandon than those that are not. Therefore, a General Linear Model (GLM) to test for factors that differentiate the two groups was conducted.

A maximal GLM was fitted using whether the nest was parasitised as the response variable and group size, number of cells at the start of the study period, site and time of season if abandonment occurred as explanatory factors. Interactions between terms were also included in this maximal model. The number of larvae and nest size (cells) were highly correlated ($r^2 = 0.55$) so only nest size was included in the model. Terms were dropped from this full model via backward elimination, until further removals led to a significant ($p<0.05$) increase in residual deviance. Significance was assessed using a Chi-square test. The resulting minimal model contained only significant terms. The significance levels quoted are for subtraction from the minimal model. All significant effects are included in the results, along with any significant two-way interactions.

3.3.d Producing survival function estimates

The factors affecting abandonment rate can best be modelled by looking for differences between nests that survived the study period and those that did not. Abandonment data was analysed using survivorship analysis, where abandoned nests are counted as having "died" and non-abandoning nests "survived". The Kaplan-Meier method (Kaplan and Meier 1958) is able to account for censored data; where the ultimate fate of samples is unknown because censusing finished whilst the samples were still alive at the end of the census period. Estimates that do not consider such censored data are prone to underestimate the survival rate. The

programming language R was used along with the "**survival**" package (Therneau and Lumley 2003) in order to fit Kaplan-Meier models to field data.

The Kaplan-Meier method of estimating survival makes several assumptions; that the actual act of taking the census does not significantly affect survival; that the factors being studied that affect survival do not change in their effect through the study period, and that abandonment happened at the times specified.

The census technique used in this study was designed to be as non-invasive as possible, with no contact with the nests other than brief observation. Parasite attack occurs during a relatively short time period in the nest cycle of *P. dominulus*, so it is hoped the second assumption has been fulfilled. The analysis will factor in the time in the season that abandonment occurred and any interactions with other factors will be noted in regards to this assumption. Daily abandonment censusing, combined with the definition of nest abandonment as nests being unoccupied for 3 consecutive censuses, satisfy the third assumption.

3.3.e Does *P. semenowi* attack affect the overall survival rate of *P. dominulus* nests?

Survival analysis determines whether there are significant differences in abandonment rates between parasitised and unparasitised nests over the entire census period. A parametric regression on the data was performed using the "R" **survreg** function from the **survival** package (Therneau and Lumley 2006). Maximal models were fitted to the data, with group size, number of cells at start of census period, whether a nest was parasitised and site as explanatory factors. The total number of cells and total number of larvae were highly correlated ($r^2 = 0.55$) so only number of cells was used in the analysis. Interactions between terms were also fitted but are included in results only if they were significant. Progressively simpler models were fitted, testing them for significant differences in deviance against the previous model using chi-square tests. This process was continued until the minimal adequate model was reached, where any removals of factors caused significant

reduction ($p<0.05$) in the explanatory power in the model. The significance of each term (or relevant interaction of terms) was calculated by adding each term individually to the minimally adequate model. Only significant interactions of terms are reported in the results.

Models were initially fitted using an exponential error model and the minimal adequate model compared with parameterised Weibull, Gaussian, Logistic, Lognormal and Log-logistic distributions to determine which model produced the lowest residual deviance. The Weibull model produced the lowest deviance, with a scale parameter value of 0.685. This model was then used to estimate mean survival of parasitized verses non-parasitized nests.

3.3.f Is the initial violent parasite attack a significant cause of abandonment?

I define the "parasite attack" as the initial violent usurpation event that occurs when the parasite first takes over the nest. The survival analysis above determines whether there are significant differences in abandonment rates between parasitised and unparasitised nests over the entire census period, but does not test whether the actual parasite attack, specifically, causes abandonment. To do this, the rate of abandonment of parasitised nests, *on the day of attack*, was compared to the daily abandonment rate of nests not exposed to the parasite. If the act of the parasite attacking caused abandonment of nests, then one would expect the numbers of attacked nests abandoning to be significantly larger than expected values generated from the background, non-attacked, abandonment rate.

The method used to perform this test is as follows:

1. The estimated "background" daily abandonment rate for each day of censusing was obtained from the Kaplan-Meier survival model and, using bootstrapping with re-sampling (n=1000), a bootstrapped distribution of mean daily abandonment rate was generated. The mean value of this distribution was taken to be the average daily abandonment rate.
2. I generated the expected number of parasite nests that abandon in one day, should they be abandoning at the same rate as unparasitised nests, by multiplying the average daily rate obtained from step 1 by the total number of parasitised nests.
3. The observed number of parasitised nests that abandoned within 1 day of parasite attack were then tested for significant difference (Chi-Square $p<0.05$) against the expected numbers generated above.

The bootstrap distribution was used to generate expected daily abandonment values, in preference to taking the mean of the daily estimates from the Kaplan-Meier model, as it allowed estimation of the population mean without assuming the original sample distribution was normally distributed. If abandonment did not occur at a uniform rate over the study period, any skew would still be reflected in the bootstrapping distribution obtained.

In order to fully safeguard against any seasonal effects on abandonment rates, another analysis using the highest recorded daily abandonment rate from the Kaplan-Meier model to generate "maximum" expected numbers of abandoning and non-abandoning nests was used to test for significant differences of the observed numbers from these more conservative numbers.

3.4 Results

3.4.a Timing of Parasite Attack

The majority of parasite attacks took place within a window of 3-4 weeks in both 2004 and 2005 (Figure 3:1), during the time when nests were reasonably large (mean number of cells = 34 ± 21) and contained larvae. Only 4 (2004) and 2 (2005) parasitised nests were discovered in the entire period prior to the start of the abandonment surveys and are not included in these analyses.

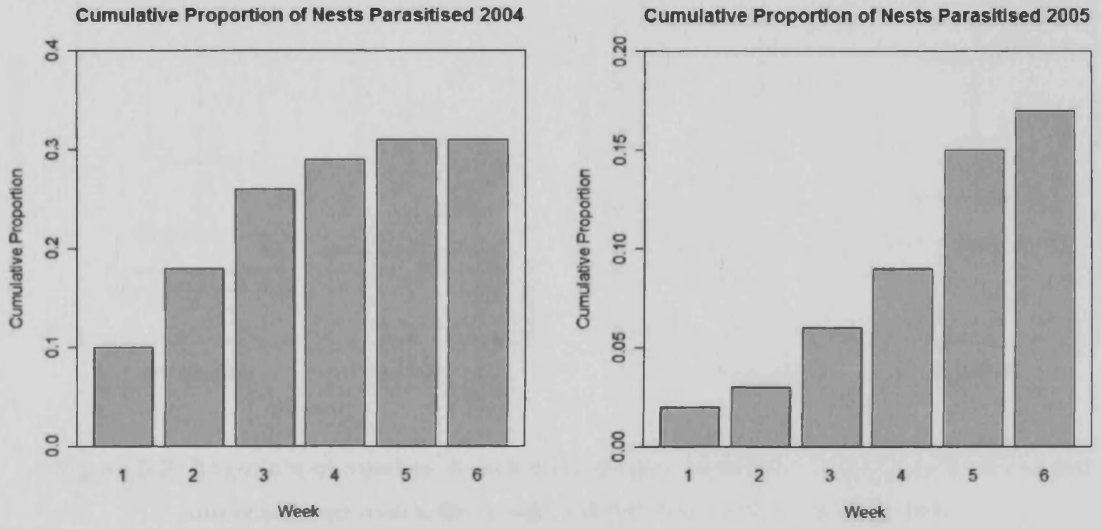

Figure 3:1: Cumulative Proportion of nests parasitised in 2004 and 2005. Week 1 began on 24th March in 2004 and 23rd March in 2005 (total nests, n=196 in 2004, n=130 in 2005).

31% (in 2004) and 18% (2005) of nests studied in the abandonment analysis were attacked by the parasite *P. semenowi*. As *P semenowi* parasites were not individually marked, it is possible that any parasites that subsequently left a parasitised nest, for example after host abandonment (personal observation n=2 incidences) could have parasitised another, therefore increasing the apparent parasitism levels.

3.4.b Differences between parasitised and unparasitised nests

In a GLM, the number of host foundresses was significantly higher on parasitised nests than unparasitised nests, as indicated in Figure 3:2 (mean no of foundresses; parasitised nests 4.68 ± 2.89, unparasitised nests 3.93 ± 2.50, GLM; effect of number of foundresses χ^2 = 0.737 , $p<0.05$). Likewise, parasitised nests had significantly higher initial numbers of cells than unparasitised nests, as indicated in Figure 3:2 (mean no. of cells; parasitised nests 43 ± 19 cells, unparasitised nests 31 ± 20 cells, GLM; effect of number of cells χ^2 = 2.22 , $p<0.05$).

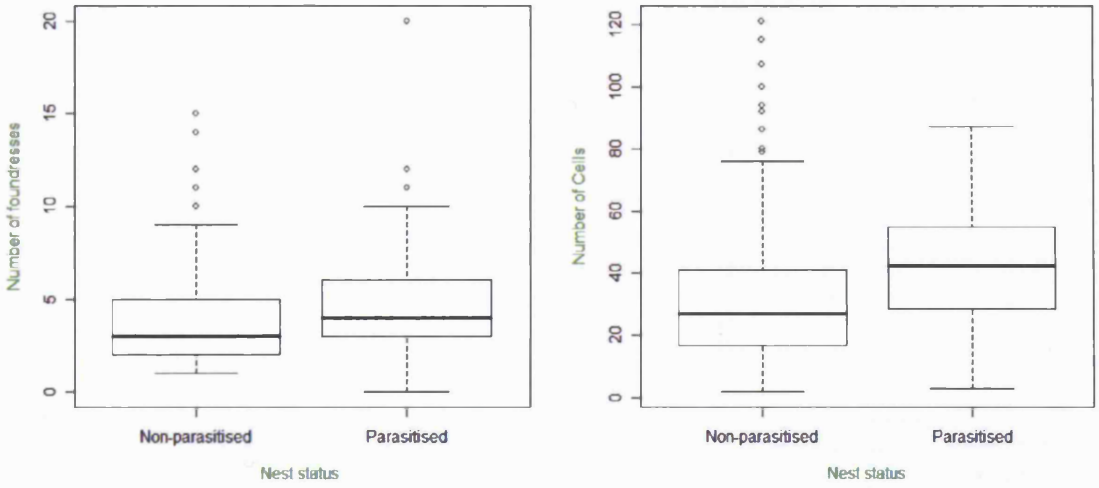

Figure 3:2: Box-plots of number of foundresses and number of cells of parasitised and unparasitised nests. Bars indicate the 95% confidence intervals.

There were a number of significant interaction terms. The number of foundress x number of cells interaction indicated that very large nests with large group sizes were perhaps more able to remain unparasitised (GLM; effect of interaction χ^2 = -2.53, $p<0.05$). The number of foundress x Farm 2005 site interaction was probably an effect of the larger upper range of foundress numbers observed at this site, meaning nests on average had larger group sizes than the other sites. The difference in group size between parasitised and unparasitised nests at the Farm 2005 site was therefore less pronounced (GLM; effect of interaction χ^2 = -2.52, $p<0.05$). Likewise, the Number of Cells x Farm 2005 interaction indicated that the difference in the number of cells between parasitised and unparasitised nests was less pronounced at the Farm 2005 site (GLM; effect of interaction χ^2 = -2.66, $p<0.01$).

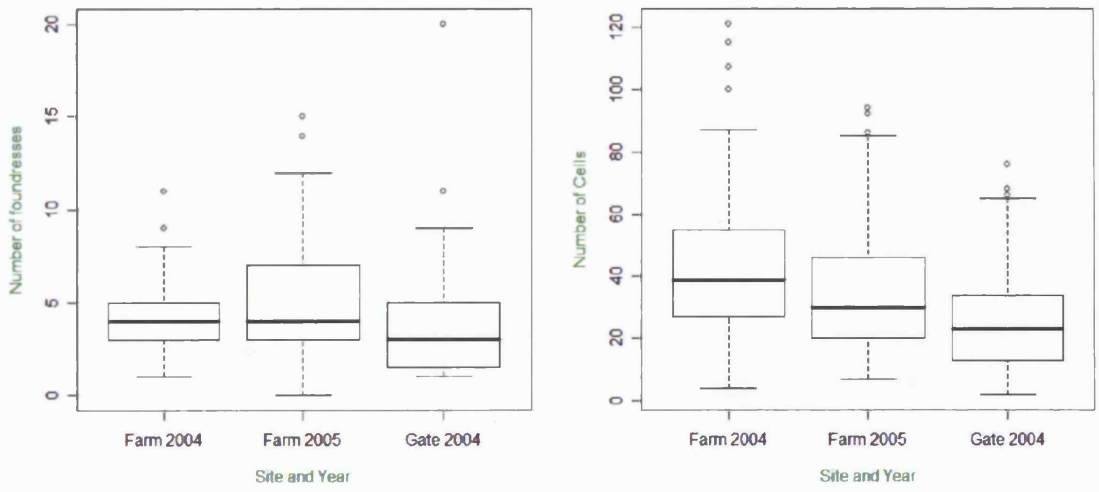

Figure 3:3: Box-plots of number of foundresses and number of cells of nests from the three sites. Bars indicate the 95% confidence intervals.

3.4.c Does *P. semenowi* attack affect the overall survival rate of *P. dominulus* nests?

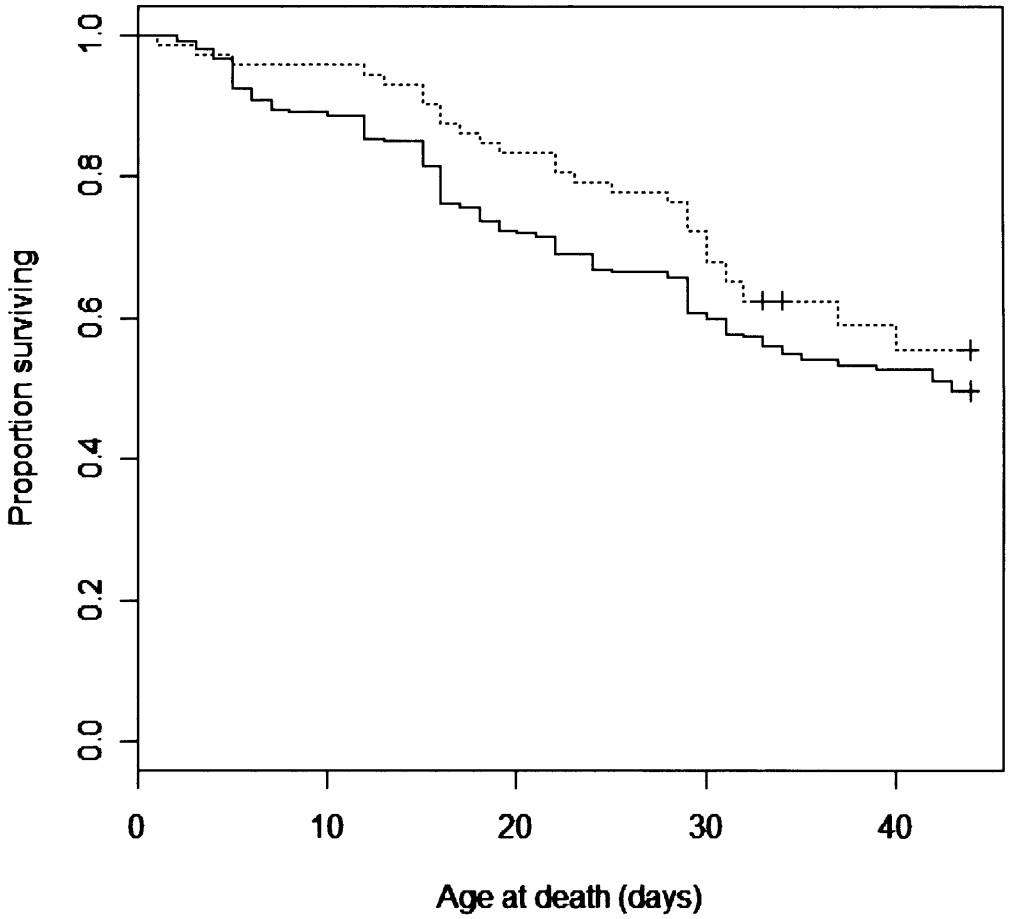

Figure 3:4: Kaplan-Meier survival curves for parasitised (dotted line) and unparasitised (unbroken line) for all sites and years. The "Age At Death" purports to the day of abandonment relative to the start of the censusing period.

Figure 3:4 shows the survival curves for parasitised and unparasitised nests. As suggested by the curves, no significant effect of parasitism on survival was found in the parametric regression ($p>0.05$). Site, the initial number of cells, the initial number of foundresses, and the interaction between the initial number of cells and foundresses all caused significant effects on the rate of nest abandonment,

independent of parasitism. All other factors and interactions did not cause significant effects ($p>0.05$). Significant main terms and their effects are discussed below.

3.4.c.i. Site

The survival rate in 2005 at the farm site was significantly greater than either site studied in 2004 (χ^2 = 2.41, p<0.001). The Gate and Farm sites in 2004 did not differ significantly in their survival rates.

3.4.c.ii. Number of foundresses

As the initial number of foundresses increased, survival increased (χ^2 = 4.74, p<0.001). The survival curves examining number of foundresses (Figure 3:5) clearly shows that small nests (1-2 foundresses) were more prone to abandoning than larger nests (3+ foundresses).

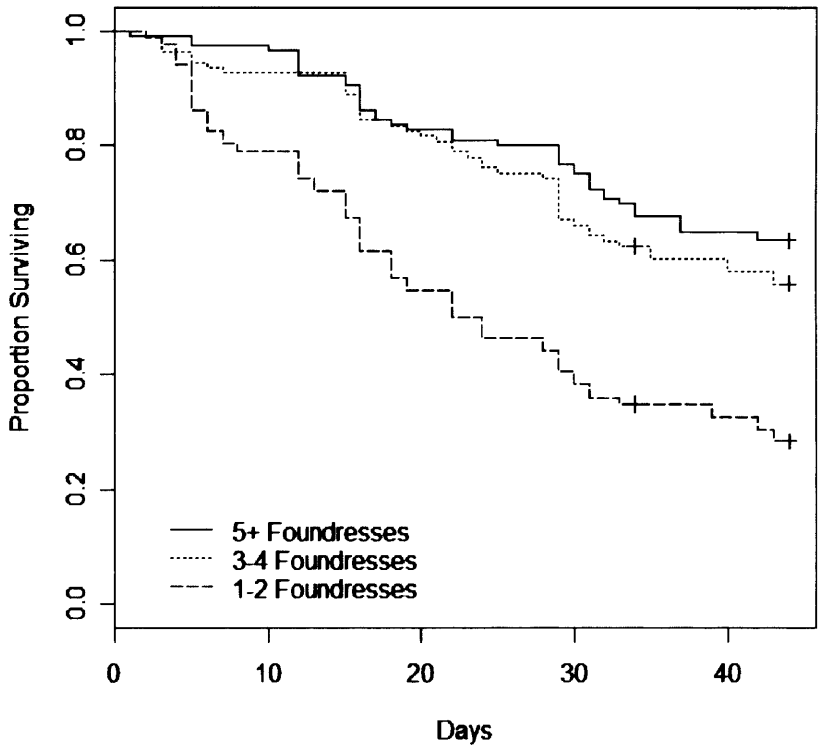

Figure 3:5: Kaplan-Meier survival curves for different ranges of initial number of foundresses. Time in Days is relative to start of the census period.

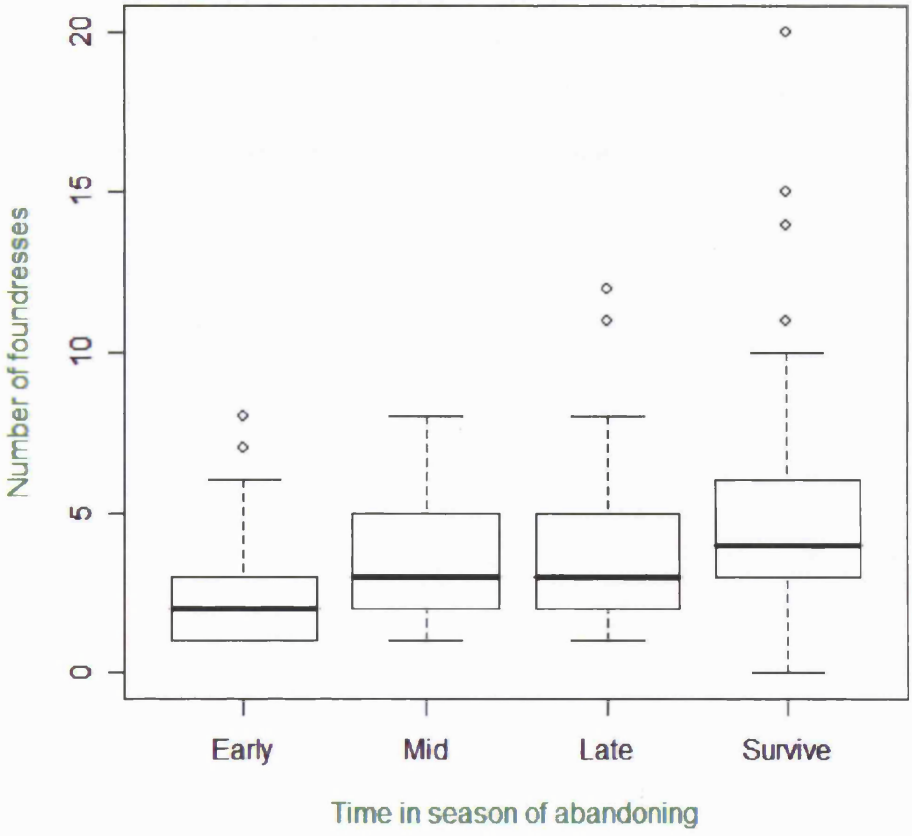

Figure 3:6: Box-plots of the initial number of foundresses present on abandoning nests at different times in the study. The time in the season is grouped into 2 week periods, "Early" being 0-2 weeks, "Mid" 2-4 weeks, "Late" 4-6 weeks and all nests that survived being grouped into "Survive". Bars indicate the 95% confidence intervals.

This effect can be appreciated by examining the mean initial number of foundresses on abandoning nests at different times of the season. Nests with more foundresses at the start of the census period survived, on average, later into the season, with the mean initial number of foundresses on nests that survived the study period being the greatest of all (Figure 3:6).

3.4.c.iii. Number of cells

As the initial number of cells that a nest had at the start of the study period increased, the survival rate increased (χ^2 = 3.54, p<0.001). Nests with higher initial cell numbers tended to survive longer into the season

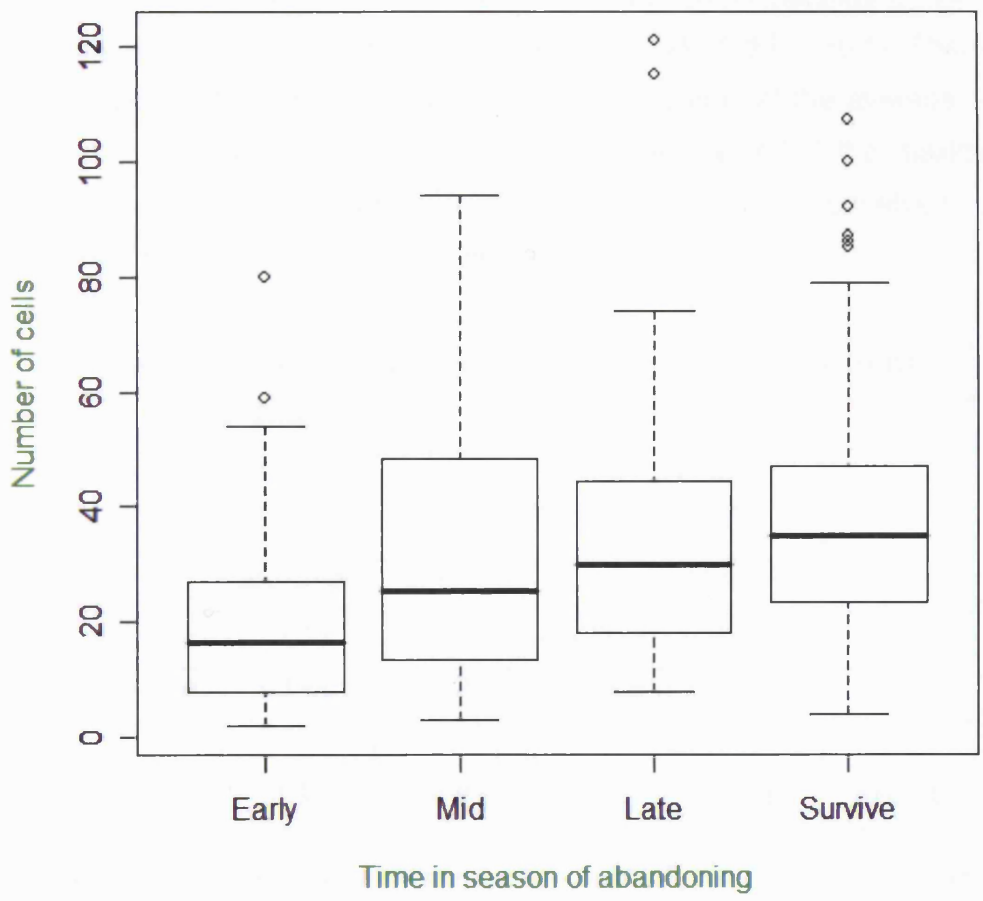

Figure 3:7: Box-plots comparing the initial number of cells of a nest versus time in the season it abandoned. Time in season is the same as in Figure 3:6. Bars indicate the 95% confidence intervals.

The interaction term between number of cells and number of foundresses was also significant (χ^2 = -4.01, p<0.001). The effect of this interaction suggests that when a nest had a large number of cells and foundresses, it did not obtain the full benefits

towards survival of each, rather there was a limit on their effects on survival when combined.

3.4.d Does parasite attack itself cause abandonment?

In chi-square tests using the bootstrapped daily survival rates to generate expected abandonment numbers, the observed numbers were significantly higher than expected (χ^2 =6.59, 1 d.f., $p<0.05$), as shown in Table 3-1. When using the maximum daily survival rate, observed abandonment after parasite attack was not significantly different from the expected rate (χ^2 = 2.29, 1 d.f., $p>0.1$). Therefore the null hypothesis that nests attacked by parasites abandon at the average observed "background" rate can be rejected, but cannot be rejected if the maximum rate observed is used. The maximum rate of abandonment observed occurred immediately following a period of heavy rainfall.

Average Daily Survival Rate			**Low Daily Survival Rate**		
	D.A.R.			**D.A.R.**	
	0.012			0.054	
	observed	*expected*		*observed*	*expected*
abandon	8.00	0.71	*abandon*	8.00	3.19
survive	51.00	58.29	*survive*	51.00	55.81
Chi-Sq=	**6.59**	**p<0.025**	**Chi-Sq=**	**2.28**	**p>0.05**

Table 3-1: Chi-Square test tables comparing observed nest abandonment observed immediately after parasite attack (< 1 day) and generated expected values for average and low survival rates. D.A.R. gives the Daily Abandonment Rate estimates used to generate the expected values.

These results indicate that there is an increase in host abandonment immediately after *P. semenowi* attack. The response is low overall, with only 13.6% of attacked nests abandoning, but is an order of magnitude larger than the average rate of abandoning in non-parasitised nests (1.2%). It is possible that this increase in

abandonment is due to the parasite killing the hosts, but no dead or damaged wasps were found near nests with lone parasites on them. Because wasps included in the abandonment census were not marked, it was impossible to determine the fate of abandoning host wasps. However, in 2004, 2 out of 3 marked individuals from a nest used in other experiments were observed to have re-nested after abandoning due to parasite attack (Almond 2004, unpublished data). Also, in personally observed usurpations in the field (n=3) and the laboratory (n=9, unpublished data), I never saw *P. semenowi* kill or seriously injure a host foundress.

3.5 Discussion

3.5.a Summary of results:

Area of Focus	Description of results obtained
Timing of *P. semenowi* attack	The majority of attacks occur within a 3-4 week period in late springtime
Level of parasitism in the populations studied	18% of nests studied were parasitised in 2005 and 31% in 2004
Parasitised nest characteristics	A GLM analysis showed parasitised nests had more foundresses and cells. In 2005, the difference between parasitised and unparasitised nests in number of foundresses was less and in number of cells was more.
Factors affecting survival	Parasitised and unparasitised nests did not show any significant difference in their survival rate. More nests abandoned later in the study than in the first 2 weeks. Nests with larger numbers of cells or foundresses had better chance of surviving, although this effect was decreased if they had both. Nests studied in 2005 had a higher survival rate than in 2004.
Changes in mean nest characteristics as the season progressed.	Those nests with larger numbers of foundresses or cells at the start of the study survived longer. Smaller nests abandoned earlier in the season.
Does abandoning occur in response to parasite attack?	Nests attacked by *P. semenowi* abandoned immediately in greater numbers than the background rate of abandonment, but not the maximum observed rate of abandonment

Table 3-2: A description of the main findings of the studies performed in this chapter.

3.5.b Level of parasitism in the populations studied

Parasitism by *P. semenowi* on *P. dominulus* affected 31% of nests in 2004 and 18% in 2005 during this study. Another study of this population (Shreeves *et al.* 2003) reported a lower level (21/156, 13.4%) but that study was not concerned primarily with parasite activity, censuses were generally made only once a week during the morning when parasite activity is rare, and manipulation of nests may have influenced observed parasitism levels. Also, the parasitism rate reported was only for parasite attack after the manipulations of nests. Other authors have reported *P. semenowi* parasitism rates exceeding 20% in other areas of the Mediterranean (Cervo and Dani 1994). Where it has been studied, therefore, parasitism by *P. semenowi* is quite high.

3.5.c Timing of parasite attack

In my study, *P. semenowi* began parasitizing nests over a month after the season had started, when nests had many cells (mean 34 ± 21) and had begun to rear the first brood. In six weeks, over 20% of nests had been attacked by the parasite. At the end of this period, many nests had produced their first workers, making usurpation a much more difficult task. *P. semenowi* attacks in a window of opportunity between nest initiation and worker emergence. Usurpation just prior to worker emergence seems to occur in all 3 *Polistes* social parasite species (Cervo and Dani 1994; Cervo and Turillazzi 1996; Zacchi *et al.* 1996).

Attacking too early, when larvae are not present, might cause foundresses to abandon as the costs incurred abandoning would be relatively small. The parasite, apparently lacking the ability to produce workers, would be left with an empty nest and no prospect of reproduction on that nest. It will also have incurred the risk of being injured by the host in the initial attack and the energetic costs of attack. Also, the earlier the parasite attacks, the more likely it is that the nest would fail before worker emergence; by attacking just prior to worker emergence the parasite avoids this risk of failure.

Leaving attack until later in the season would guarantee the availability of large nests with many larvae and foundresses to exploit, but increases the chance of

host workers being present and hence the number of wasps to have to fight for control of the nest. In the laboratory, *P. semenowi* can invade nests with first workers present, however, so increased nest defence might not be the only reason why the parasite does not seem to attack later in the season (Zacchi *et al.* 1996). Time constraints on producing brood before the season ends increase as the season progresses, so parasites should be expected to time their attack to ensure they have sufficient time to rear their brood.

3.5.d Differences between parasitised and unparasitised nests

Parasites seemed to choose nests that were larger, both in terms of group size and physical size of the nest (Figure 3:2). A previous study of this population found a similar result, with *P. semenowi* attacking both naturally smaller and experimentally reduced groups at a lesser rate (Shreeves *et al.* 2003).

Large nests with more foundresses provide the parasite with a greater workforce: more foundresses in the early stages of parasitism and more workers later on. Large nests would allow the parasite to rear many of its offspring at once, fully exploiting the productivity of its host. Foundresses on larger nests may also be more likely to stay on the nest if parasitised, as they can still benefit from rearing any host offspring present, as long as the benefits *per capita* increase as nest size increases. These benefits may outweigh the costs of leaving the nest to pursue other reproductive options.

By choosing larger nests, *P. semenowi* may not only guarantee a larger number of host workers to help rear its own young, but also cause foundresses to stay and help as well. However, there are problems with this explanation. Larger groups have been shown to be more successful at re-nesting in *Polistes bellicosis* (Strassmann *et al.* 1988). One might therefore expect the nests parasitised by *P. semenowi*, which have higher numbers of foundresses on average, to be more likely to abandon. Also, the number of larvae per foundress has been shown to be constant in the population of *P. dominulus* studied here (Shreeves *et al.* 2003).

P. semenowi therefore benefits from both increased reproductive capacity and improved probability of survival by selecting larger nests.

3.5.e Factors affecting nest survival

No significant difference in overall survival between parasitised and non-parasitised nests was shown in this study. This finding agrees with another study of this population where nests parasitised by *P. semenowi* were no more likely reach the worker stage than unparasitised nests (Shreeves *et al.* 2003). Parasitised nests in that study were less likely to be abandoned than unparasitised nests, although only weekly censuses were used to assess nests and so could have missed some parasite related abandonment.

Larger groups (3+ foundresses) were more likely to survive than smaller ones (1-2 foundresses), as shown in Figure 3:5. Smaller groups, on average, failed earlier than larger groups (Figure 3:6). This result may suggest that there is an insurance-based advantage of helping, "survivorship insurance (SI)". SI occurs when the chance of nest failure (and hence total reproductive failure) due to the deaths of all individuals in a group decreases as group size increases (Nonacs 1991; Queller 1994; Nonacs and Reeve 1995). My results do indicate that larger nests survive longer into the season and that the initial group size at the start of the study had an effect on survival. The study of this population mentioned previously failed to detect SI through analysis of experimentally reduced groups and from natural group sizes (Shreeves *et al.* 2003).

Nests with larger numbers of cells had significantly increased survival rates. As group size is strongly correlated with nest size, this is not surprising and the result probably reflects that increased foundress numbers increase the number of foundresses that work on building the nest in the initial phases of nest building.

3.5.f Host immediate response to parasite attack

Despite the apparently high rate of parasitism, abandonment of nests immediately following parasite attack (< 1day) was only a minority event (8/68 (11.8%) parasitised nests overall), even if it did occur at a rate significantly above the natural abandonment rate (1.2% per day). Although there were no signs of foundress death in these 8 nests, it is possible that the parasite killed all the foundresses. Mortality

due to *P. semenowi* has not been observed in any previous study or in my own personal observations in both laboratory and natural colonies (Demolin and Martin 1980; Mead 1991; Cervo and Dani 1994; Zacchi *et al.* 1996).

It is possible that my daily censuses did not record all parasite attacks so that a few attacked nests might have been recorded as having abandoned independent of parasitism. This would mean my data would underestimate the abandonment response to the initial parasite attack. As parasitism occurred in the late morning and afternoon, my censuses should have detected any parasites that stayed on the nest for more than a few hours. Only parasites that left the nest in a relatively short time would have been undetected.

3.5.g Why is host abandonment of *P. semenowi* so rare?

My results show that there might be evidence that abandonment rate increases following parasite attack, but overall abandonment in response to parasitism is rare. There are many reasons why a nest may not abandon;

3.5.g.i. Evolutionary Lag

Abandonment may be adaptive, but there might not have been sufficient time for genes coding for abandonment to have appeared and spread in the population as a result of selection (Davies and Brooke 1988; Rothstein 1990). Parasitism seems relatively common, and the cost of parasitism, a severe reduction or total loss of further reproduction, seems high.

It is possible that the level of parasitism encountered in the study population does not represent the normal parasitism rate. The sites used were chosen because they allowed study of a large number of nests with relative ease. This high density of nests may sustain higher numbers of parasites than less densely aggregated populations. In areas of rare or no parasite incidence, there would be little selective pressure to develop specific anti-parasite measures. The possibility of migration and interbreeding between high and low incidence populations in the study area might further reduce the fixation of abandonment behaviour in response to parasite attack.

3.5.g.ii. Equilibrium

Abandoning and subsequently re-nesting incurs the costs to the host foundresses of the energy spent in finding building materials, time spent making the new nest and energetic costs of rearing new brood. Also, hosts that re-nest are constrained in the amount of time left in the season to reproduce in.

If the hosts stay, they can rear any host larvae present on the nest. The hosts are potentially able to rear some reproductive offspring from the existing host offspring present at the time of parasite attack (see Chapter 5). The costs for *P. dominulus* hosts therefore may not be as high as in hosts of avian brood parasites, such as the European cuckoo, where no host reproduction is observed on parasitised nests (Davies 2000). There is a chance, as well, that the parasite may die or abandon the nest, allowing the host to resume reproduction (Mead 1991). In such cases the hosts would re-inherit the nest along with any workers that hatched out during parasite dominion.

It could be, therefore, that the costs of rejection of the parasite via nest abandonment outweigh those of acceptance (Zahavi 1979). Possible factors involved in the costs and benefits of staying or abandoning are discussed below.

3.5.g.ii.i. Conspecific usurpation

It could be that the level of conspecific usurpation in the population is important in the evolution of abandonment as an anti-parasite strategy. An invading wasp could be related to foundresses, so that costs of staying are much less than those of accepting a non-related parasite. If behaviour adapted towards invaders has been selected upon primarily in the context of conspecific attack, or nest joining, then one might not expect a strong abandonment response.

This might be especially true for larger group sizes, where conspecific usurpation is unlikely, due to increased number of defenders. In such cases, the primary response to usurpers, violent attack, is mostly successful, making any other response unnecessary. The parasite, however, can invade such nests and so would take advantage of the lack of any further defences. However, if conspecifics are

unable to invade such nests and the parasite is, then larger nests might be expected to evolve an "always abandon if usurped" counter-parasite strategy.

The usurping behaviour of *P. dominulus* conspecifics and *P. semenowi* are very similar (Cervo and Lorenzi 1996; Zacchi *et al.* 1996). It may be difficult for *P. dominulus* foundresses to differentiate the two types of usurper, making evolution of specific responses to *P. semenowi* parasitism unlikely. Recognition cues are discussed in detail in chapter 5. Parasites could potentially be differentiated by the hosts using facial markings, which differ significantly from *P. dominulus* markings. These facial markings have been shown to be used by *P. dominulus* in communicating fighting ability, so could feasibly be used in discrimination of the parasite from host conspecific invaders (Tibbetts and Dale 2004). Epicuticular odour has been shown to be involved in nestmate recognition, and *Polistes* parasites seem to have vastly reduced levels of such odours, perhaps making discrimination of the parasite difficult (Lorenzi et al. 2004).

3.5.g.ii.ii. Loss of existing brood

My data support previous studies of *Polistes sulcifer* and *P. semenowi* showing that parasites usurp more advanced nests, containing more cells with more foundresses than the population average (Cervo and Turillazzi 1996; Shreeves *et al.* 2003). These nests are also less likely to fail as the season progresses (Figure 3:5). By leaving the nest, the hosts may decrease the chance of the nest surviving long enough for their larvae to hatch out. It could be that host foundresses on parasitised nests choose therefore to stay in order to ensure the survival of larvae present in the nest.

3.5.g.iii. Other Costs of abandonment

Abandonment of nests and brood therein incurs temporal costs and often results in smaller clutches and lower offspring survival in birds if the parent re-nests (Davies and Brooke 1989; Rohwer *et al.* 1989). Renesting pairs of Least Bell's Vireo (*Vireo bellii pusillus)* that had deserted parasitised nests have a much lower seasonal

productivity than unparasitised pairs (Kus 2002). It may be that abandonment of nests in *Polistes dominulus* is too costly an activity and that the potential benefits of direct reproduction (Cervo *et al.* 1990; Mead 1991; Dapporto *et al.* 2004) outweigh the benefits of abandonment.

3.5.h Alternative methods of investigating how parasite attack affects abandonment

I chose to observe natural abandonment because I was interested in seeing whether abandonment is used as a counter-parasitism strategy under natural conditions. The methods used did not control other factors that might affect abandonment and rely on accuracy in observing parasite presence and any subsequent abandonment, as well as accurate recording of other factors which might affect abandonment.

A future approach might be to observe *P. semenowi* attack of *P. dominulus* nests in the laboratory, noting any abandonment that occurred as a result. This approach echoes experiments with avian hosts examining response to visual contact with stuffed cuckoos (Davies *et al.* 1996). Presenting dead *P. semenowi* adults might initiate a response but may not present the behavioural and olfactory cues associated with a natural attack. Olfaction has been shown to be important in acceptance or rejection of nest-mates in *Polistes* (Lorenzi *et al.* 1997).

The above method could be adapted to use live *P. semenowi*. In the field, live specimens would have to be constrained in some way to stop them escaping during experimental usurpation, which again could affect parasite behaviour. Laboratory colonies could be maintained and parasites introduced, but doing so removes any external cues the hosts might use to assess the costs and benefits of staying or abandoning. All these approaches also take away the choice of which nest to parasitize from the parasite and rely on non-biased nest selection by the investigator.

3.6 Summary

P. *semenowi* attack on *P. dominulus* nests does seem to increase the rate of nest abandonment over the background rate, within 24 hours of parasite attack. After this time, parasitised nests on average fare as well, or better than unparasitised nests, perhaps because parasites chose "higher quality" nests with larger groups and more cells. Host abandoning due to parasite presence was therefore only detected in the period immediately following parasite attack and the host decision to abandon is probably made at that point. It is likely that the costs associated with abandonment, coupled with possible benefits of staying to raise remaining related brood or inheriting the nest should the parasite leave, lead to abandonment occurring at the low rates observed in this study.

Chapter 4. **Aggression in Social Animals**

4.1 Reproductive dominance

Reproductive dominance has been well studied in many eusocial animal groups; in such groups, only a few individuals gain direct fitness by rearing their own reproductively capable offspring. The majority of group members forgo reproduction and help rear the offspring of the dominant (Chapter 1, Hamilton 1964).

The position of reproductive dominant is therefore a valuable one, as not only does the dominant produce its own offspring, but it also gets to harness the effort of the rest of the group in rearing them. As with any resource, there is conflict over who takes the role of this dominant position. Individuals compete to obtain reproductive dominance, and in many cases those that fail to become dominant immediately compete with other subordinates to be next in line to the dominant. Should the dominant die, this subordinate takes over. In many instances, therefore, dominance hierarchies form with individuals competing for position in the hierarchy, forming a queue for the dominant position (Pardi 1948).

4.1.a Dominance Hierarchies

The formation of dominance hierarchies is commonplace (Gust 1995; Cummins 1996; Heinze 2004; Field et al. 2006) and can be organised through:

- *Direct physical aggression*: where dominance position is determined by fighting ability, often related to an individual's physical condition and size e.g. *Polistes* (Pardi 1948), male Musk Ox (Wilkinson and Shank 1976), male Narwhals (Silverman and Dunbar 1980). The majority of this category concerns male-male competition for access to females (Clutton-Brock and Parker 1992), although female-female aggression to attain nest dominance or

monopolise resources also commonly occurs in several species such as birds (Slagsvold and Lifjeld 1994) and *Polistes* species (Tibbetts and Dale 2004).

- *Ritualised aggression:* where dominance position is determined by contests which are physically demanding and rely on individual physical condition, but individuals do not run the risk of mortality through competing e.g. Red Deer (Clutton-Brock and Albon 1979), Crested Ibis (Li et al. 2004), Hissing Cockroaches (Carrel and Tanner 2002).
- *Fitness "badges"*; the size of a cue present on individuals correlates to individual fitness and incurs a proportional cost upon the owner. Dominant individuals bear the "best" badge (e.g. Harris sparrow (Rohwer and Rohwer 1978), *Polistes dominulus* (Tibbetts and Dale 2004)). Contests are therefore avoided as long as the badge is an honest signal of fitness.
- Conventions e.g. age-based queuing in *Liostenogaster flavolineata* (Field et al. 2006) and *Polistes exclamans* (Strassmann and Meyer 1983). Again, contests are avoided as long as the convention is upheld by every group member.

4.1.b Dominance in Polistes

In *Polistes* species, only females compete for dominance on the nest. Control of reproduction is normally taken by a single female (Theraulaz et al. 1990; Queller et al. 1997). Elements of each of the different ways of initiating and maintaining a dominance hierarchy (see 4.1.a) seemingly occur in *P. dominulus*, and the social parasite *P. semenowi* has adaptations which may serve to help it integrate into the hierarchy (Table 4-1):

Method used	***Polistes dominulus***	***Polistes semenowi***
Direct physical aggression	Fighting for dominance position at nest initiation (Tibbetts and Reeve 2000)	Aggressive usurpation (Zacchi et al. 1996); thicker mandibles and stronger forelegs than hosts (Cervo and Dani 1994)
Ritualised aggression	Non-violent actions such as licking, mounting and antennal stroking are used to dominate and reproductively suppress subordinates (Theraulaz et al. 1990).	Reportedly uses the same dominance behaviours as dominant hosts (Mead 1991)
Fitness "badges"	Black clypeal markings indicate fighting ability and may be used in establishing dominance (Tibbetts and Dale 2004)	Facial markings on *P. semenowi* consist of more black pigmentation, speculatively this might be a "super" signal of high fighting ability.
Conventions.	Possible age-based queue of workers in *P. annularis* (Queller et al. 1997)	Parasite arrives before worker emergence so obtains dominance through the convention.

Table 4-1: Methods of dominance hierarchy establishment and maintenance in *Polistes dominulus*, with possible devices *Polistes semenowi* uses in exploiting this hierarchy.

In *P. dominulus*, the control of individuals lower in the dominance hierarchy is assumed to be mostly through physical means (Tibbetts and Reeve 2000; Cant et al. 2006), although some studies suggest that aggression subsides after initial establishment of the hierarchy and a chemical based dominance system is used thereafter (Sledge et al. 2001; Dapporto et al. 2004). Within the dominance hierarchy, stereotypical interactions occur between individuals who are higher ranked (dominant) than other individuals (subordinate). The interactions can be concerned directly with the maintenance of the hierarchy or with behaviours such as transfer of food and liquids between individuals (trophallaxis). This chapter is concerned with the former, agonistic interactions.

4.1.c Aggression in Polistes

Some social behaviour can be thought of as aggression (Moyer 1983); actions concerned with the retention or acquisition of access to a resource, in this case rank in the dominance hierarchy and hence inheritance of the breeding position upon the nest. Previous studies on *Polistes* have defined the following acts as social actions concerned with aggression (Gamboa et al. 1990; Reeve and Nonacs 1992; Cant et al. 2006).

- *Darting*: Wasp rapidly moves towards another wasp, antennae pointing at it, the act often ending in an attempted bite, usually followed by rapid retreat of the initiator. Previous studies have implicated it in regulation of subordinate activity on the nest (Sumana and Starks 2004), as well as in dominance interactions (Cant et al. 2006).
- *Lunging*: Movement towards another wasp, without biting but using the body to impact, with no retreat by the initiator.
- *Grappling*: Grabbing another wasp using forelegs, often pushing it across the nest.
- *Mounting*: Climbing upon another wasp, often accompanied with licking of its body.

These interactions usually are most intense between individuals of similar ranks in the hierarchy (Cant et al. 2006), with dominant individuals maintaining their place in the queue whilst subordinates assess the fitness of their superior with the ultimate aim of supplanting any dominant which is less fit (Field et al. 1998).

4.1.d Dominance behaviour of P. semenowi

Little has been written about the behaviour of *P. semenowi* on *P. dominulus* nests after the initial usurpation event. Mead (1991) observed the aggressive interactions on one nest for 5 hours, but reported only overall aggression rates. The level of aggressive interactions described seems low and the aggressive actions were not defined precisely. Other authors state that the parasite behaves in a similar way to host dominants in terms of aggression (Cervo and Dani 1994). Studies on other Polistes brood parasites, such as *P. sulcifer*, also note that the patterns of parasite dominance behaviour resembles that of host alpha dominants but did not quantitatively examine the issue (Turillazzi et al. 1991). An in-depth analysis of dominance behaviour, in comparison to host behaviour is still needed for *P. semenowi*.

Subordinate aggression on nests parasitised by *P.* semenowi has also not been studied. If observed aggression of subordinates differs on parasitised nests, there are several possible explanations;

1. Parasite dominance behaviour differs from host dominant behaviour, which in turn affects host subordinate behaviour. An alternative is that parasite presence alters host subordinate behaviour, but not necessarily through direct behavioural interaction with the parasite.
2. *P. semenowi* selects nests whose subordinates differ in aggressive behaviour from other *P. dominulus* nests. For example, parasites may attack nests that have less aggressive individuals in order to be faced with less resistance when usurping.

The two hypotheses can be examined by observing host behaviour both in presence and absence of the parasite, in comparison to unparasitised nests. If the parasite's behaviour influences host subordinates, its removal should cause the hosts to revert back to "typical" unparasitised host behaviour, whereas if subordinates are inherently less aggressive, no change in aggression should occur after parasite removal.

4.1.e Polistes semenowi and hierarchy exploitation

One aim of this chapter is to examine how *Polistes semenowi* affects intra-nest aggression and foraging effort of *P. dominulus* subordinate foundresses. As a parasite, unrelated to its hosts, one might expect *P. semenowi* to attempt to maximise host effort in rearing its brood. Some forms of aggression have previously been found to influence subordinate activity (Sumana and Starks 2004). Aggression by the parasite might therefore be a mechanism for stimulating their effort, as depicted in Figure 4.1-1 (Reeve and Gamboa 1983; Reeve and Gamboa 1987; Sumana and Starks 2004).

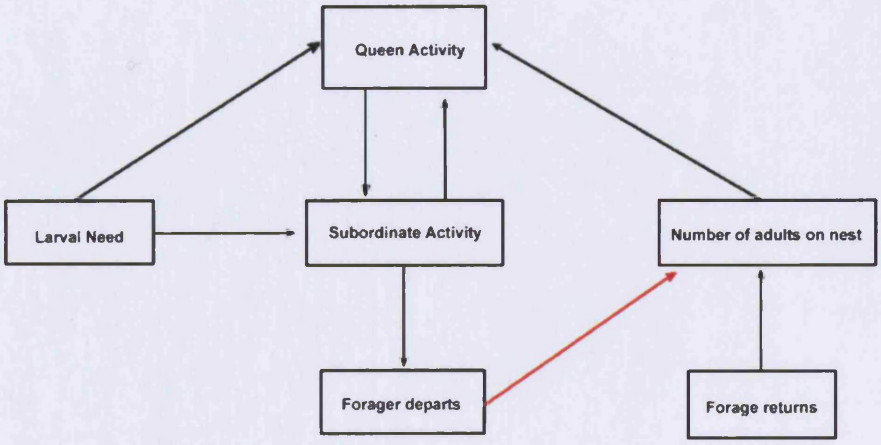

Figure 4.1-1:A possible social feedback system for regulating colony activity in *Polistes* (adapted from Reeve and Gamboa 1987). Red arrows depict negative feedback.

If parasites do force hosts to maximise effort, then both parasite activity (in actions that stimulate host effort) and host foraging should be expected to differ from

unparasitised dominant activity and the effort of unparasitised subordinates respectively.

4.2 Aims

The main aims of this chapter are as follows:

- Determine whether *P. semenowi* has a similar behavioural profile as the dominant individual on unparasitised *P. dominulus* nests.
- Investigate whether aggression between individuals on parasitised nests differs from that on unparasitised nests.
- Investigate whether parasite behaviour influences subordinate foraging activity.

4.3 Methods:

A total of 21 parasitised nests and 21 unparasitised controls, matched for group size, nest size and brood composition, were videotaped in the field in 2003 and 2004 (see Appendix 5 and Chapter 2). For each nest, 100+ minutes of footage were watched for aggressive interactions before and after removal of the parasite or the dominant foundress on control nests. In addition, a series of twenty or more censuses was performed at 30 minute intervals in the field, to assess which wasps were present on the nest in order to determine the identity of the dominant of unparasitised nests, as done previously (Cant and Field 2001). As with other studies, dominance was found to be related to increased time on the nest, and supports the use of time-on nest as a predictor of the identity of dominant individuals used in this thesis (Cant and Field 2001; Cant et al. 2006).

The following aspects of behaviour were recorded from the videos:

- Time of arriving at and leaving the nest
- Donor and recipient of aggressive acts.

As with previous studies the proportion of time that each foundress spent on the nest was highly variable (Cant and Field 2001; Cant et al. 2006),. Usually the dominant individual is almost constantly present on the nest, giving it more time to initiate and receive social actions (Cant *et al.* 2006). This means that comparison of aggression must be done by standardising the data in respect to time on the nest. As in previous studies, the "Total Aggression Rate" (Cant et al. 2006) was therefore calculated as the number of aggressive acts per minute of presence on the nest. An aggression "rate" was recorded separately for each of the 4 types of aggression mentioned previously (see p. 83), both for acts initiated and received, and the total rate calculated by weighting each act equally and summing the acts.

4.3.a Data sources

Data on both aggressive behaviour and time spent on the nest were recorded for 21 parasitised and 21 unparasitised nests, comprising individual observations for 126 marked foundresses and 21 *P. semenowi* adults. Nests were filmed between 15 and 21 days after the parasite arrived on the nest, to ensure sufficient time for parasite brood to be present on the nest. The parasite or host dominant was then removed on the evening of the first day of filming and the nest filmed again on the next suitable day after removal.

Filming occurred between the hours of 10am to 5pm, when wasps were most active. Filming occurred only on clear, calm days to ensure wasp activity. Parasites and control dominants were removed in the evening of the first day of filming, leaving 12 hours between removal and subsequent filming of the nests after removal. Filming before and after removal usually occurred on subsequent days unless weather conditions did not permit it. Filming after removal occurred in these cases on the next fine day, at most within 2 days. Further details of procedures followed can be found in Chapter 2.

4.3.b Statistics

Data were tested for normality with the Shapiro-Wilks test. If data were found to be non-normal, and log transformation of data did not improve normality, non-parametric tests were used. Otherwise, a General Linear Mixed Effects Model was used (see 4.3.c).

For paired data comparisons, such as from matched parasite-control nest pairs, the Wilcoxon signed rank test with matched pairs was used. Large outlying data values can sometimes lead to false positives in t-tests that assume unequal variances; the Wilcoxon rank sum test does not suffer as much from such effects. This test is thought to be more powerful than the analogous matched pair t-test when assumptions of normality of the distributions cannot be satisfied (Daniel 1978).

4.3.c General Linear Mixed Effects Model

For analysis using General Linear Mixed Models (GLMMs), a log (Y+1) transformation was used on total, dart and lunge aggression data to improve the fit of the residuals in the model to assumptions of normality. Data on proportion of time spent on the nest were arcsine transformed to improve the fit. As mounts and grapples were rarer events than lunges or darts (Figure 4.4-1), individuals were coded binomially, with "0" for no acts initiated or received and "1" indicating the act was initiated or received. Subsequently, GLMMs for grapples and mounts were fitted with binomial errors. GLMMs for total aggression, darts and lunges were fitted with normal errors ("REML" Genstat 8.0). Nest identity was fitted as a random effect in all models. In both models of aggression rates and proportion of time spent on the nest, the following were fitted as fixed effects in a maximal model:

- Whether an individual was the dominant
- number of cells
- group size (including the parasite)
- whether the nest was parasitised
- the year of study
- the relative day in the season on which the nest was filmed

Also, for analysis of the proportion of time an individual spent on the nest, the total and individual aggression rates received and performed were also included as explanatory variables.

Fixed effects were dropped from the full model via backward elimination, until removal of any terms remaining led to a significant decrease in the explanatory power of the model ($p<0.05$). This was assessed using a Wald statistic that is asymptotically distributed as χ^2 (Genstat 8.0). Each term was separately added to the minimally adequate model to assess its significance. Relevant two-way interactions were also tested in the presence of main effects in this way, but were not included in the results unless significant.

4.4 Results:

The majority of aggression initiated and received were either darts or lunges (Figure 4.4-1).

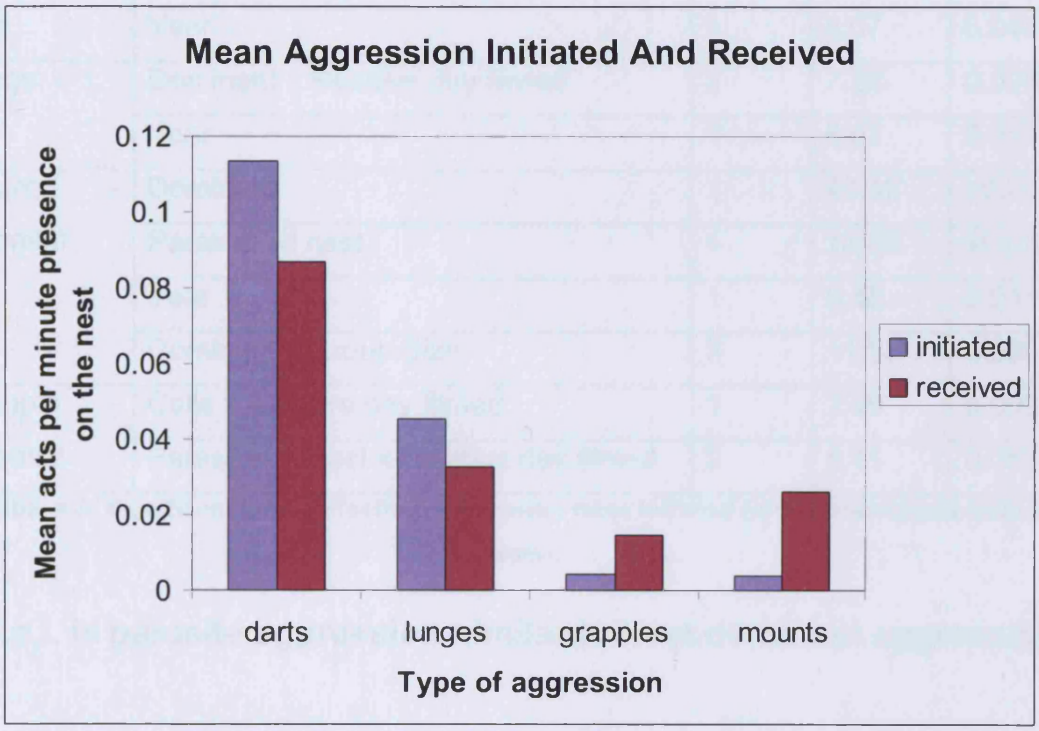

Figure 4.4-1: Mean aggression rates observed for all nests

4.4.a Aggression initiated

Total aggression rates initiated were highly variable between individuals (mean ± standard error = 0.044 ± 0.092 acts per minute per individual, range: 0.000-0.815).

After following the procedure detailed in section 4.3.c, significant factors affecting lunge, dart, grapple, mount and total aggression initiated were determined via GLMM models. The significant terms in each GLMM are given in Table 4-2:

Aggression Initiated	Fixed Term	d.f.	Wald Statistic	p
Total	Dominant x Parasitised Nest	3	14.34	0.002
	Year	1	9.11	0.003
Dart	Year	1	4.07	0.044
Lunge	Dominant x Relative day filmed	2	7.36	0.025
	Year	1	6.07	0.014
Mount - binomial	Dominant	1	40.42	<0.001
	Parasitised nest	1	12.65	<0.001
	Year	1	6.48	0.011
	Dominant x Group Size	2	11.58	0.003
Grapple - binomial	Cells x Relative day filmed	1	7.49	0.006
	Parasitised Nest x Relative day filmed	2	6.11	0.047

Table 4-2: Significant terms affecting aggression rates initiated (n=147 individuals from 42 nests).

4.4.a.i. Is parasite aggression similar to host dominant aggression?

P. semenowi adults initiated significantly less total aggression than host dominants (GLMM, Wald = 14.34p = 0.002, see Table 4-2). However, in terms of individual aggression rates, the parasite did not initiate darts or lunges at a significantly different rate to host dominants (GLMM, Dominance x Parasitised interaction, both p>0.05). Parasites were also not significantly more likely to initiate mounts or grapples than host dominants (GLMM Dominance x Parasitised interaction, p>0.05 for both). Therefore, the parasite's individual aggressive behaviours did not differ much from *P. dominulus* dominant behaviour for the majority of individual aggression initiated, but overall it was less aggressive.

4.4.a.ii. Is aggression initiated different between individuals on parasitised nests and unparasitised nests?

Overall aggression rates initiated per individual did not differ between parasitised and unparasitised nests (GLMM, effect of nest being parasitised, $p > 0.05$). When comparing individual aggressive actions initiated, individuals on parasitised nests were more likely to initiate mounts (GLMM mounts initiated, effect of nest being parasitised $\chi^2 = 12.65$, $p < 0.001$). Dart and lunge rates, and the likelihood of initiating grapples, did not differ between individuals on parasitised and unparasitised nests (GLMM, $p>0.05$).

4.4.a.iii. Other factors affecting initiated aggression

Dominant individuals were more likely to initiate mounts than subordinates (GLMM, Dominance effect, $p<0.05$). Dominant individuals in larger groups were more likely to initiate mounts than those in smaller groups (GLMM, Dominance x Group size effect, $p<0.05$). Dominant individuals (both parasite and host dominants) initiated fewer lunges as the season progressed (GLMM, Dominance x Relative day filmed effect, $p <0.05$).

In 2005, the initiated total aggression rate, as well as the dart and lunge rates, were higher than in 2005 than 2004 (GLMM, effect of year, all $p<0.05$). Individuals in 2004 were more likely to initiate mounts than those in 2005 (GLMM, effect of year, $p<0.05$).

The likelihood of individuals initiating grapples on large nests increased as the season progressed (GLMM, Nest size x Relative day filmed effect, $p<0.05$). The likelihood of an individual initiating a grapple decreased as the season progressed on parasitised nests (GLMM, Parasitised x Relative day film effect, $p<0.05$).

4.4.b Aggression Received

Total aggression rates received were highly variable between individuals (Figure 4.4-1, mean ± standard error = 0.050 ± 0.097 acts per minute per individual, range: 0-1.017).

After following the procedure detailed in section 4.3.c, significant factors affecting lunge, dart, grapple, mount and total aggression rates received were determined via GLMM models. The significant terms in each GLMM are given in Table 4-3.

Aggression Received	Fixed Term	d.f.	Wald Statistic	p
Total	Dominant	1	5.18	0.023
	Group size x Relative day filmed	1	5.03	0.025
	Group size x Year	2	10.67	0.005
Dart	Year	1	7.34	0.007
	Year x Dominant	2	6.6	0.037
	Group size x Relative day filmed	1	4.59	0.032
Lunge	Dominant x Group size	1	8.25	0.004
	Group size	1	13.07	<0.001
	Year	1	22.74	<0.001
	Dominant	1	19.72	<0.001
Mount	Parasitised Nest	1	9.9	0.002
	Year	1	7.24	0.007
Grapple	Group size x Year	2	6.04	0.049

Table 4-3: Significant terms affecting aggression rates received (n=147 individuals from 42 nests)

4.4.b.i. Do parasites receive aggression at a similar rate to host dominants?

Parasites received the typical rate of aggression that an equivalent *P. dominulus* dominant foundress received from subordinates on an unparasitised nest. There were no significant differences in either total or individual aggression rates received between the parasite and host dominants (GLMM, Parasitised x Dominant interaction, $p>0.05$ for all).

4.4.b.ii. Is aggression received differently between individuals on parasitised nests and unparasitised nests?

Individuals on parasitised nests were more likely to receive mounts than those on non-parasitised nests (GLMM; effect of nest being parasitised, $p<0.05$). Individuals on parasitised nests did not have significantly different total or individual dart or aggression rates received than those on unparasitised nests (GLMM; effect of nest being parasitised, $p>0.05$ for all). Likewise, individuals on parasitised nests were no more likely to receive grapples (GLMM; effect of nest being parasitised, $p>0.05$).

4.4.b.iii. Other factors affecting aggression received

Dominant individuals received lower total aggression rates than subordinates (GLMM; dominance effect, $p<0.05$). Dominant individuals received a lower lunge rate than subordinates (GLMM; dominance effect, $p<0.05$). Dominants in larger groups received more lunges than those in smaller groups (GLMM; dominance x group size effect, $p<0.05$). Dominant individuals did not receive darts at a significantly different rate to subordinates, nor were they more likely to receive mounts or grapples (GLMM; dominance effect, $p>0.05$ for all). However, subordinate individuals received a higher dart rate in 2005 than in 2004 (GLMM; dominance x year effect, $p<0.05$).

In 2005, the received dart and lunge rates were higher than in 2005 than 2004 (GLMM, effect of year, all $p<0.05$). Individuals in 2004 were more likely to receive mounts than those in 2005 (GLMM, effect of year, $p<0.05$). However, individuals in larger groups received more total aggression than large groups in 2004, and were also more likely to receive grapples (GLMM; group size x year effect, $p<0.05$ for all). In 2005, the initiated total aggression rate, as well as the dart and lunge rates, were higher than in 2005 than 2004 (GLMM, effect of year, all $p<0.05$). Individuals in 2004 were more likely to perform mounts than those in 2005 (GLMM, effect of year x group size interaction, $p<0.05$).

Individuals in larger groups received a lower lunge rate than those in smaller groups (GLMM; group size effect, $p<0.05$). Finally, individuals in large groups received relatively more darts and total aggression as the season progressed (GLMM, group size x relative day filmed effect, $p<0.05$ for all).

4.4.c After Removal

4.4.c.i. Does removal of the parasite cause increased intra-nest conflict?

Individual aggression rates changed on nests parasitised by *P. semenowi* after removal of the parasite. Total aggression rates per minute of time on the nest, per nest mate were calculated for aggression initiated and received by subordinates. These rates were calculated before and after removal of the parasite or host dominant. Both total and individual aggression rates before and after dominant removal for parasitised and unparasitised nests were then tested individually using the Wilcoxon signed ranked test (see section 4.3.b).

The grapple and mount rates received decreased significantly after removal of the parasite (mean grapple rate received; before = 0.0015 ± 0.0019 acts/foundress/minute, after = 0.0001 ± 0.0003 acts/foundress/minute, Wilcoxon signed rank test $V= 36$, $p = 0.014$, mean mount rate received; before = 0.0036 ±

0.0040 acts/foundress/minute, after = 0 ± 0 acts/foundress/minute, Wilcoxon signed rank test V = 55 p = 0.006). Individual aggression rates initiated and received did not differ significantly after removal of the dominant on unparasitised nests (Wilcoxon rank sum test, p>0.05 for all). All other individual rates of aggression initiated and received did not alter significantly (p>0.05).

There was no significant difference between mean total aggression rates received before and after dominant removal in both parasite and host nests (Wilcoxon signed rank test, p>0.05 for all). Likewise, no difference was detected between total aggression rates initiated before and after dominant removal on both parasitised and unparasitised nests (Wilcoxon signed rank test, p>0.05 for all).

4.4.c.ii. Time on nest

There was no evidence that host subordinates worked harder on parasitised nests than on unparasitised nests. Individuals on parasitised and unparasitised nests did not significantly differ in the time they spent off the nest (GLMM, effect of parasitism, χ^2 = 0.10, p = 0.757). Also, no differences were detected between activity of subordinates on parasitised and unparasitised nests, or between the host dominant and the parasite, in the proportion of time spent on the nest (GLMM with nest as random effect, effect of parasitism x dominant effect χ^2 = 2.33, p = 0.098).

Dominant individuals spent more time on the nest (GLMM, effect of dominance χ^2 = 13.93, p < 0.001). Individuals in larger groups spent more time on the nest than those in smaller groups (GLMM, effect of group size χ^2 = 4.24, p < 0.04).

Levels of aggression initiated and received by individuals were negatively correlated with the amount of time they spent upon the nest. Individuals that received a higher total aggression rate spent less time on the nest (GLMM, effect of received total aggression rate χ^2 = 27.12, p < 0.001). When considering individual aggression, wasps that initiated more lunges or received more darts spent more time off the nest (GLMM, effect of individual aggression, lunges initiated χ^2 = 6.5, p =

0.011, darts received $\chi^2 = 12.44$, $p < 0.001$). All other factors and interactions had an insignificant effect on the time an individual spent on the nest ($p>0.05$).

4.4.d Summary of Aggression Results

Direction of Aggression	Aggressive Act	Relative difference of parasites versus host dominants	Relative difference of dominants versus host subordinates	Relative difference - individuals on parasitised nests versus unparasitised nests	Difference after parasite/ dominant removal	
					Control nests	Parasitised nests
Received	Total	0	-	0	0	0
	Dart	0	0	0	0	0
	Lunge	0	-	0	0	0
	Mount	0	0	+	0	-
	Grapple	0	0	0	0	-
Initiated	Total	-	+ (for host)	0	0	0
	Dart	0	0	0	0	0
	Lunge	0	0	0	0	0
	Mount	0	+	+	0	0
	Grapple	0	0	0	0	0

Table 4-4: A summary of results obtained in GLMM analysis of aggression ("+" = increased, "-" = decreased, "0" = no difference)

4.5 Discussion

4.5.a Does the parasite have a different aggression profile than host dominants?

This study largely supports previous observations of *P. semenowi*, that found that the parasite exhibits individual aggressive behaviours at levels similar to an equivalent host alpha foundress, staying on the nest with most of its activity concerned with dominating subordinates (Demolin and Martin 1980; Mead 1991; Zacchi et al. 1996; Lorenzi et al. 2004).

However, parasites initiated significantly less total aggression towards host subordinates than host dominants, but did not significantly differ in the total aggression they received (Table 4-4). The rates of all individual aggressive interactions, both initiated and received, were not significantly different between the parasite and host dominants (Table 4-4).

I hypothesised that use of aggressive threats might have been one of the methods by which *Polistes* parasites control their hosts (Cervo et al. 1990; Cervo and Lorenzi 1996; Fanelli et al. 2005). Whilst the profiles of each *individual* aggressive behaviour were quite similar between parasites and host dominants, the parasite seems to be less aggressive overall, which contrasts to observations that aggression profiles were similar (Mead 1991; Zacchi et al. 1996). Aggressive threats therefore are not used any more by the parasite than by host dominants to control subordinates, and the reduction in parasite aggression might even suggest that the parasite is less reliant on such threats.

One explanation for reduced parasite aggression is that somehow the parasite placates host subordinates in other ways, perhaps with either pheromones, or with bribes of reproduction. However, if this were the case, then one might expect host subordinate aggression towards the parasite to be reduced on nests with *P. semenowi* than towards the host dominant on unparasitised nests. Because the total aggression that host dominants and the parasite received were not significantly different, this alternative hypothesis does not seem to be supported.

The parasite may be somehow better at either withstanding or performing aggression. The parasite's morphological adaptations such as its thickened cuticle and mandibles may serve this purpose. If the costs of receiving host aggression are lower for parasites, then they may be less inclined to retaliate to challenges from subordinates than host dominants. Alternatively, if aggression performed by the parasite is somehow more effective, it may need to perform less aggression overall to achieve the same effect as a host dominant.

4.5.b Aggression on parasitised nests

Individuals on parasitised nests were significantly more likely to initiate and receive mounts than those on unparasitised nests (Table 4-4). Mounting behaviour is believed to be concerned with dominance interactions, possibly with maintaining an individual's place in the dominance hierarchy (Tibbetts and Reeve 2000; Nonacs et al. 2004). Dominant individuals were more likely to perform mounts, suggesting a role in control of subordinates. The increased mounting behaviour on parasitised nests suggests that the profile of aggression is different to unparasitised nests, host foundresses on nests parasitised by *P. semenowi* may devote more effort to maintaining their place in the dominance hierarchy.

If the total level of aggression exhibited by a dominant was an indicator of condition, then a reduction in aggression might trigger increased dominance interactions down the dominance hierarchy, as such a reduction may indicate the demise of the dominant is forthcoming. Each foundress might then benefit from testing those wasps immediately above them in the hierarchy, to assess whether they could potentially usurp their position and become closer to taking over the dominant position (should the dominant die or leave). The parasite, therefore, by exhibiting less aggression, may in turn incite increased levels of dominance interactions amongst host subordinates. This hypothesis could be tested further by experimentally slowing dominant host individuals and examining subordinate response to reduced aggression from the dominant.

It has been suggested for *P. atrimandibularis* parasitizing *P. biglumis bimaculatus* that the host "dominant", rather than the parasite, inhibits ovarian development in host workers. The parasite needs the presence of a host foundress

in order to maintain reproductive control over workers (Cervo and Lorenzi 1996). If this were the case in *P. semenowi*, then the patterns of aggression exhibited on the nest might be different, with the highest ranking subordinate exhibiting the most aggression, rather than the parasite queen. I could not control for an individuals rank within the dominance hierarchy as I only knew the identity of the dominant individual. Cant et. al's (2006) study of individual aggression found that aggression rates decreased down the hierarchy. My study considered subordinates as a whole, meaning that the aggression rate variance between subordinates could have swamped any differences between dominant and subordinates. A further study, determining dominance rank of host subordinates on parasitised nests might reveal whether the parasite queen does use a host foundress as a means of controlling host reproduction.

4.5.c If the parasite is removed, does subordinate aggression change?

There are two feasible explanations as to why host subordinate behaviour differs between *P. semenowi* parasitised and unparasitised nests. The parasite may be directly causing the behaviour, through its aggression or presence, or the parasite may have chosen to attack a nest where foundress behaviour differs in some way from other nests (section 4.1.d). These two hypotheses can be differentiated by the predicted effect of removal of the parasite: if parasites were using aggression as a means to increase colony productivity, or if parasite presence was correlated to increased aggression amongst subordinates, removal of the parasite should trigger a change in subordinate aggression rates. If parasites chose nests with subordinates that were different in some way in terms of their behaviour, no change in behaviour would be observed if the parasite was removed.

No significant differences were detected between total aggression rates before and after removal on parasitised or unparasitised nests after removal of the parasite or host dominant (see Section 4.4.c.i). There was, however, a significant decrease in the rate of mounts and grapples received by subordinates, on parasitised nests, after parasite removal. There was no equivalent decrease on unparasitised nests. Subordinates on parasitised nests performed more mounts than

those on unparasitised nests prior to removal. The reduction in mount rates received after parasite removal suggests that the parasites' presence affected host subordinate behaviour, rather than the parasite having had selectively attacked groups with inherently different levels of aggression.

It is possible that the time between parasite removal and behavioural recording (>9 hours) may not have been long enough for subordinate foundresses to adjust their aggression rates accordingly. I chose to record nests the day after removal to limit the number of nests lost through abandonment or foundress mortality before collection for molecular analysis. Recordings of nests a longer time after removal of the parasite may have given a more accurate picture of changes in aggression post parasite removal.

4.5.d Behaviours affecting time spent on nest

Dominant individuals (both parasite and host) spent the most time on the nest. The rate of total and darting aggression an individual received was correlated with the time it spent off the nest, with more time spent off the nest by individuals who received more aggression (section 4.4.c.ii). Darting behaviour has previously been implicated in activity regulation on *P. fuscatus* colonies (Sumana and Starks 2004). It was found in Sumana and Starks study (2004) that inactive workers received significantly more darts than active workers, and responded to the dart by increasing their activity. Active workers receiving darts also switched from one activity to another, suggesting that darts are not only involved in initiating activity, but also regulation of what activity is carried out. Foraging for food and building material occurs off the nest, in studies of food limitation in *Polistes fuscatus* and *P. dominulus*, more time was spent off the nest when food was scarcer, suggesting a link between foraging and time spent off the nest (Armstrong and Stamp 2003; Nadeau and Stamp 2003). Therefore, darts may be used as a method of controlling which individuals forage and also regulate the effort they make in terms of time spent off the nest.

4.5.e *P. semenowi* and host foraging

It has been suggested that *Polistes* parasites might increase productivity of their hosts, compared to subordinates on unparasitised nests, by "overworking" them (Lorenzi et al. 1991). In my study, individuals that received more aggression spent more time off the nest and hence, more time foraging. *P. semenowi* initiates significantly less aggression than host dominants on unparasitised nests, with no difference in dart rate (see Table 4-4). There was no difference in the proportion of time spent off the nest by subordinates from parasitised and unparasitised nests, so even though the parasite performs less aggression, subordinates still seem to work as hard as those on unparasitised nests (see section 4.4.c.ii).

An alternative hypothesis is that the dominant individual on a nest may not dictate the work rate of subordinates, despite monopolising reproduction (Jha et al. 2006). Jha et al. found that removal of the dominant did not significantly alter activity levels and that in most cases worker activity initiated colony activity in *Polistes instabalis* and *P. dominulus*. Because the parasites reduced aggression in comparison to host dominants had little effect on colony foraging activity, my results may add support to Jha's hypothesis. An alternative hypothesis is that the parasite queen rules "by proxy", influencing a "dominant" host individual which, through its aggression, controls subordinate activity. Further study of aggression dynamics between different ranked individuals on parasitised nests is needed to test such hypotheses.

4.6 Summary

The role of aggression in dominance of its host by *P. semenowi* still warrants further research. Subordinate aggression levels on parasitised nests differ only from unparasitised nests in increased likelihood of initiating and receiving mounts. Subordinates on parasitised nests may be diverting more effort towards dominance interactions, reflecting increased effort by subordinates towards advancing or maintaining their place in the dominance hierarchy. *P.* semenowi aggression profiles are not significantly different from host dominants' but the reduced levels of aggression exhibited by the parasite could be in some part responsible for increased mounting by the host subordinates. Further study into the dynamics of aggression amongst such subordinates on parasitised nests may provide further insight into the parasites strategy, including the possibility that the parasite relies on a high ranked host subordinate to dominate other subordinates.

Because no difference in foraging effort was found between subordinates on parasitised nests and unparasitised nests, the increased subordinate effort in dominance interactions (mounting) does not seem to significantly affect foraging effort. The lack of difference between subordinate work effort on parasitised and unparasitised nests suggests that parasites do not increase their subordinate's work rate compared to subordinates on unparasitised nests. Possible reasons for this could be either an inability to control colony activity (Jha et al. 2006), allowing a host dominant to control colony activity or because subordinates are already working at a maximal rate on control nests.

4.7 Further Work

There are many avenues of future research into aggression that might prove interesting. Is dart rate truly a mechanism by which subordinates regulate colony activity? This could be studied by observing subordinate behaviour immediately after receipt of a dart. Another possible method to investigate the role of darting might involve inactivation of colony members. If some subordinates are chilled to reduce their activity, one might expect increased darting from remaining members as an attempt to increase their activity. A similar approach might be used to investigate whether the decreased aggression initiated by the parasite cause increased mounting between the subordinates. If a host dominant is chilled, and hence reduces its initiated aggression rates, the effect on the activity of its subordinates could be studied and compared to behaviour observed on nests parasitised by *P. semenowi*.

Recent studies on clypeal facial markings and their role as a badge of dominance status could be expanded to include *P. semenowi* (Tibbetts and Dale 2004). Are the exaggerated black clypeal markings in the parasite a part of its dominance strategy? If so, manipulation of its mark might be expected to change the behavioural profile of its subordinates.

Chapter 5. **Differential Feeding**

5.1 Introduction

In this chapter I seek to examine whether host foundresses on nests parasitised by *P. semenowi* are truly deceived by the parasite, or whether they have evolved adaptations to favour related brood over brood of the parasite. When the parasite attacks, the nest contains only host brood, but *P. semenowi* usurps the position of the dominant host foundress and begins laying its own eggs (Cervo 2006). Soon, the nest contains both new parasite brood and host brood laid prior to attack. The host foundresses might be expected to discriminate against parasite brood by giving more care to host brood, or even by destroying parasite brood. In order to do this, the hosts have to have the ability to distinguish related from unrelated brood within the nest. In this section I discuss evidence supporting *P. dominulus* kin recognition abilities and whether host foundresses might be able to discriminate against parasite offspring. The ways in which the host may discriminate are then examined, along with possible parasite counter-adaptations. Finally, I discuss host reproduction on parasitised nests, as a way for the host foundresses to obtain direct fitness benefits by staying on the nest.

5.2 Kin Recognition in Polistes

Kin recognition, the ability to recognise conspecific relatives, has been an area of much study in evolutionary biology (Fletcher and Michener 1987; Gamboa et al. 1991; Hepper 1991; Gamboa 2004). A brief synopsis of kin recognition in *P. dominulus* is given in the Introduction chapter.

Kin recognition takes various forms in *P. dominulus*. Adult-adult recognition concerns the recognition of nest mates versus non-nestmates, and possibly discrimination of relatives from non-relatives. Adult-brood recognition is similarly concerned with kin versus non-kin recognition and the discrimination of nest-mate

from non nest-mate brood. Adult wasps may encounter such non-nestmate brood after usurpation by conspecifics or interspecific parasites, where a non-nestmate takes over the dominant, reproductive position (Gamboa *et al.* 1992; Cervo and Lorenzi 1996; Cervo 2006). I now discuss the nature of kin recognition in *P. dominulus* and whether there is a basis for discrimination of parasite brood from host brood.

5.2.a Recognition Cues

Studies in temperate *Polistes* species have elucidated that the main recognition cues used are present on both the nest and on the wasps themselves (Pfennig et al. 1983). They are probably chemical (olfactory) in nature (Pfennig et al. 1983), rather than visual, tactile or auditory, although some evidence of visual cues being used as an indicator of fighting ability might suggest that visual cues play a role in recognition (Tibbetts and Dale 2004). Several investigations have manipulated chemical cues, either by creating a homogenous environment so that foundresses do not differ in chemical cues, or by washing the nest so that cues are removed (Gamboa et al. 1986; Singer and Espelie 1992). In all cases nestmate recognition was subsequently disrupted.

It is important to differentiate between the proximate and ultimate origin of recognition odours, as the way odours are acquired could be important for the hosts in discriminating against the parasite. The odours are ultimately derived from both the environment (nest building materials, food etc.) and from genetic components of foundress secretions. The cues themselves consist of both endogenous and exogenously acquired chemicals (Pfennig et al. 1983; Gamboa et al. 1986; Dani et al. 1996). Both endogenous and exogenous sources could feasibly be of environmental or genetic origin. For example, genetically based odours deposited on the nest by the dominant individual could be acquired exogenously by nestmates. Gamboa et al.'s study (1986) where colonies of *P. fuscatus* were raised in identical conditions found that initially, nestmates did not discriminate against non-nestmates, *if* the wasps had been recently exposed to a common environmental odour. If the

females were isolated from such odours for several days, discrimination did occur (Gamboa *et al.* 1986). The author suggested that the common exogenous environmental odours had decayed during isolation, unmasking the wasp's endogenous heritable odours.

Chemicals produced by the wasps and deposited on the nest are likely candidates for cues that could be used to discriminate between kin and non-kin, as their constituents are determined by the wasp secretions and hence, potentially, genes. Several studies of non-nestmate kin recognition in *P. fuscatus* have shown that the recognition odour is partly derived through genetic components (Gamboa 1988; Bura and Gamboa 1994). It is hard to envisage how a system whereby cues composed solely of chemicals not produced by the wasps could be used to distinguish kin from non-kin, unless colonies of related wasps procured unique specific odours through habitual and exclusive use of select areas of their habitat. In this case the environment would have to be chemically diverse enough to afford each colony a distinct odour (Gamboa et al. 1986; Gamboa et al. 2004).

5.2.b Application of wasp derived odours onto the nest surface

The endogenous secretions are spread over the epicuticle from the Dufours' Gland area during grooming (Dani et al. 1996; Sumana and Starks 2004). Behavioural observations have identified behaviours (such as abdominal 'waggling') which serve to spread endogenously produced odours over the nest surface (Dani et al. 1992). Thus endogenous cues are shared with other nestmates via the nest. These behaviours are also associated with Interspecific usurping, where the usurper spreads its own chemical secretions over the nest so that emerging, naive host workers learn the cue and subsequently identify the usurper as a nestmate (Cervo and Lorenzi 1996). The behaviours could also serve to pick up host secretions present on the nest in order to camouflage the usurper in order for it to be tolerated by host foundresses, as has been shown to occur in interspecific *Polistes* social parasites (Turillazzi et al. 2000). Cues can therefore be manipulated by the parasite, which may affect the ability of hosts to discriminate (Lorenzi 2006).

5.2.c Nestmate recognition

Nest odour is used as a template for recognition (Ross and Gamboa 1981; Post and Jeanne 1982; Strassmann 1983). Natal nest odours are likely to be a reasonable indicator of relatedness, given the high levels of skew observed in *P. dominulus* populations (Queller et al. 2000; Liebert and Starks 2006). The observation that *Polistes* foundresses return to their natal nest site after hibernation, thus allowing previous nest-mates to find each other, adds support to this idea (Klahn 1979; Strassmann 1983; Makino et al. 1987; Gamboa 1988).

Discrimination of nestmates versus non-nestmates is very much dependent on context; the costs of accepting an individual onto the nest may differ during the colony cycle (D'Ettorre et al. 2004). Early in the season, larger groups may stand a better chance of success, so unrelated individuals may be allowed to join (see Chapter 3). Later in the season, when many large brood have been produced and worker production begins, the cost of allowing an individual to join may outweigh the benefits, as outside individuals may attempt to usurp the nest and the soon to hatch workforce (D'Ettorre et al. 2004). Foundresses might therefore be more discriminatory as the season progresses. Pratte's study (1982) of *P. dominulus* foundresses, early in the season, failed to show any preference for nesting with relatives over non-relatives, although the experiment itself was perhaps not suitable for such a test (Post and Jeanne 1982). Gastreich et al. (1990) studied *Parachartergus colobopterus* and found a similar lack of ability to discriminate nestmates versus non-nestmates. It has been suggested that the difference in relatedness between the two groups may only be minor in the population studied, thus making discrimination rather arbitrary (Gamboa et al. 1991).

When *Polistes* colonies have been established and brood produced, non-nestmates become less and less likely to be tolerated on the nest (Lorenzi et al. 1997; Pratte 1997; Starks et al. 1998; Panek et al. 2001; Starks 2003). At the time of *P. semenowi* attack, invasion is met with violent defence of the nest, so host foundresses can clearly discriminate the parasite as being a non-nestmate. The parasite is soon no longer attacked more than an equivalent host dominant,

however, suggesting that it has somehow manipulated the host's nestmate recognition system (Turillazzi et al. 2000).

5.2.d Brood Recognition

In several species of *Polistes* it has been shown that foundresses can discriminate 'alien' brood from nestmate brood. In several species of *Polistes*, usurpers destroy reproductive brood (Klahn 1988; Cervo and Turillazzi 1989; Lorenzi and Filippone 2000). In the case of brood, one study has shown that Dufours' gland secretions may allow recognition of related eggs in a colony, allowing differential egg oophagy in *P. fuscatus* (Downing 1991). One study of *P. sulcifer* has shown that parasite brood have their own distinctive cuticular hydrocarbon signatures and do not mimic host signatures (Dani et al. 2004).

Host foundresses may therefore be able to discriminate between related and parasite brood, as long as *P. semenowi* brood also bear parasite specific cues.

5.3 Parent-Offspring Conflict

As well as host foundress adaptations to discriminate against parasite brood, parasite brood may themselves evolve adaptations to exploit the host. The parasite brood differ from host brood in that they are completely unrelated to their host carers. The relationship between parasite brood and host foundresses can be thought of as a special case of parent-offspring conflict (Trivers 1974).

Because parasite brood are completely unrelated to their host carers, they should be expected to be completely selfish. The selfishness may only be limited to the point where it could damage siblings (other parasite brood) on the nest. Likewise, because parasite brood should aim to maximise their own resource intake, the costs to the hosts will be high. If this is the case, selective pressure for counter adaptations against unrelated parasite offspring should occur.

5.3.a Host Response to Parasite Brood

Given the inability of the host foundresses to physically remove the adult parasite from the nest (personal observation), combined with the expected parasite indifference to host fitness, host foundresses could instead focus on fighting the parasite through actions towards the parasite brood. This could occur through:

1. Preferential feeding of host brood over parasite brood.
2. Destruction of parasite brood.
3. Laying eggs and rearing host brood in competition with parasite brood.

If the host foundresses are able to discriminate, we can therefore expect one or all of the above situations to occur in parasitised nests.

5.3.a.i. Preferential feeding

With *P. semenowi* offspring receiving care from unrelated hosts, one should expect there to be conflict. In terms of host-parasite co-evolution, one might therefore expect competing parasite adaptations and host counter-adaptations to evolve:

1. *Parasite Exploitation*: Parasite offspring maximise host effort, soliciting feeding and developing more quickly than host offspring. Observed parasite feeding rates would therefore be expected to be the same as, or higher than equivalent host offspring rates.
2. *Host preferential feeding* (of related offspring); a host counter-strategy where host foundresses preferentially feed host offspring. Observed host offspring feeding rates would therefore be expected to be higher than equivalent parasite brood feeding rates.

The observed feeding rates would therefore reflect the outcome of the evolutionary battle between parasite and host. Parasite brood are reared in nests containing both related parasite offspring and unrelated host offspring. In other systems where both related and unrelated brood are present on a resource, intense rivalry often occurs (Walls and Roudebush 1991; Godfray and Parker 1992; Kilner 2003). In species where no parental care occurs and offspring are laid on a large shared resource, selfish behaviour such as eating shared food faster than it can be assimilated, to speed up development, is observed (Godfray et al. 1991). The reasoning behind this is that although the food would support more individuals if eaten at the optimum rate, in line with peak assimilation efficiency, those that eat more are less likely to be left wanting and therefore ensure their survival. The genes for this "greediness" therefore spread at the expense of non greedy genotypes. In a similar manner, *P. semenowi* brood might be expected to fully exploit host care at the expense of host brood, especially since there is no guarantee that they will be in a nest with any related offspring (i.e. the first parasite offspring will be sharing the nest with mainly host larvae). A study of *P. dominulus* nests parasitised by *P. sulcifer* found that host

workers gave more feeding behaviour to parasite larvae, but did not control for larval size and did not examine host foundress feeding behaviour (Cervo et al. 2004).

5.3.a.ii. Mechanisms of exploitation

Parasites may exaggerate begging behaviour for food in order to maximise care, as with avian brood parasites such as cuckoos (Kilner et al. 1999; Davies 2000; Kilner 2003). The mechanism of larval begging in *Polistes* is not investigated here, but it has been suggested that auditory/ vibratory cues may be used to indicate nutritional need (Jeanne 1980). In addition, I suggest that other cues such as chemical cues passed from brood to adults via trophallaxis may also be an indicator; if host adults gauged larval nutrition via levels of 'waste' products of digestion, then a parasite brood that sequestered such products would appear malnourished and so solicit more feeding. Such waste products could, for example, be by-products of larval growth, such as amino acids or sugars present in larval trophallactic fluids (Hunt et al. 1982). An alternative suggestion would be that the secretions are nutritionally important for adult wasps (Hunt et al. 1982). They would thus be attractive, so parasites exaggerating this lure would encourage more care and possibly feeding. Inefficient assimilation of food would presumably also result in higher levels of such by-product substances.

5.3.a.iii. Brood destruction by Host Foundresses

As explained on page 110, there is potential for host foundresses to be able to discriminate between related host larvae and unrelated parasite larvae. Differential oophagy has been reported in *Polistes* as part of the mechanism of dominance determination (Pardi 1948). In *Polistes* species where conspecific usurpation occurs, usurping wasps eat the previous dominants eggs whilst allowing their own to develop (Cervo and Lorenzi 1996; Starks 1998). In *Polistes chinensis* colonies, both dominant and workers have been observed to eat workers eggs in preference to those of the queen, again suggesting an ability to discriminate (Saigo and Tsuchida 2004). *Polistes* foundresses therefore seem able to discriminate between nestmate

and non-nestmate eggs. Lorenzi and Filippone (2000) found that eggs of the social parasite *Polistes atrimandibularis* transplanted into host *P. biglumis* nests were destroyed 60% of the time, compared to destruction of 25% of control eggs (transplanted *P. biglumis* eggs transplanted elsewhere on the same nest). This result suggests that parasite eggs could be discriminated, although only 4 nests were studied. Whether eggs actually laid on the host's nests by the parasite, rather than experimentally transplanted, are eaten has not been shown; parasite actions on the host nest may disrupt discriminatory mechanisms. On the basis of studies so far, however, host foundresses may be able to discriminate and so challenge the parasite by continually destroying its eggs and larvae.

Unpublished studies by Cervo et. al. of larvae of *P. sulcifer* suggest that such larvae neither mimic the epicuticular hydrocarbon profile of host larvae, nor possess a reduced profile which could make their detection as parasites more difficult (Lenoir et al. 2001; Cervo 2006). They also purportedly found that *P. sulcifer* larvae were accepted by hosts that had not been exposed to the adult parasite (Cervo 2006). *P. sulcifer* larvae may therefore have specific, non-mimetic adaptations to ensure acceptance by hosts. *P. semenowi* larvae could also have such adaptations which ensure that they are not destroyed by host foundresses.

The threat of injury in retaliation by the parasite may deter any host destruction of brood. Parasite presence could be exerting a kind of "Mafia" effect (Zahavi 1979). However, should the parasite die or abandon the nest, one might expect hosts to remove the unrelated parasite brood and feed their corpses to their own related brood, as they are no longer being influenced by the parasite.

There could be a high cost to hosts of making recognition errors; destroying their own eggs as well as those of *P. semenowi*. *P. biglumis* foundresses presented with non-nestmate eggs also destroyed 25% of their own eggs (Lorenzi and Filippone 2000). If *P. semenowi* manipulates the host recognition system, then the possibility of recognition errors occurring may increase.

5.3.b Host Reproduction on Parasite Nests

Another aim of this chapter is to examine whether hosts reproduce on parasitised nests. One of the main concerns in the study of the evolution of sociality is the question of who, in the social group, gets to reproduce. In *Polistes dominulus*, it is usually found that a single foundress occupying the alpha dominance position monopolises the majority of reproduction (Introduction Chapter, p.15 & p.23). Some studies have explained the evolution of helping behaviour, by subordinates that do not reproduce, in terms of indirect fitness: by foregoing reproduction to help a relative breed, the subordinate indirectly passes on copies of its genes to the next generation via the offspring of its relative. However, this explanation fails to account for unrelated helpers, who do not gain such benefits (Queller et al. 2000; Liebert and Starks 2006). Subordinate foundresses on nests parasitised by *P. semenowi* are unrelated to the parasite, so if the parasite completely monopolises reproduction, they have no incentive to stay other than inheriting the nest should the parasite leave or die (Mead 1991). One way *P. semenowi* may encourage host foundresses to stay, therefore, would be to "bribe" host foundresses to stay and help by allowing them some direct reproduction.

5.4 Aims

The main aims of this chapter are to investigate the following:

- Are host foundresses exploited by parasite brood into feeding them preferentially, or does the host differentiate parasite offspring from its own and hence preferentially feed host offspring?
- Does parasite presence on the nest affect the pattern of feeding performed by the host foundresses?
- Does brood destruction occur on parasitised nests? If the parasite is removed, do hosts destroy parasite brood?
- Do hosts on parasitised nests manage to rear any of their own offspring when parasitised?

5.5 Methods

A total of 17 parasitised nests were studied (Table 5-1).

Nest	Date of parasite attack	Date filmed	Minimum no. of days parasite present before filming	Time between parasite removal and 2nd day's filming (days)	No. of host foundresses	At time of filming			
						No. of cells	No. of eggs	No. of Larvae	No. of worker
nrP4	19/04/2004	22/05/2004	33	1	2	84	14	30	7
nr59a	30/04/2004	24/05/2004	24	2	2	56	7	22	4
nr121a	28/03/2004	22/05/2004	55	1	2	99	40	33	1
59a	16/04/2004	14/05/2004	28	1	2	77	20	30	8
146	09/04/2004	24/05/2004	45	2	3	90	5	23	4
16	08/04/2004	20/05/2004	42	1	3	138	59	46	4
p42	26/03/2004	07/04/2004	12	1	1	59	19	14	3
P4	30/03/2004	20/05/2004	51	1	1	77	3	10	6
7	11/04/2004	25/04/2004	14	1	1	68	27	17	1
s172	22/04/2005	08/05/2005	16	1	2	55	17	9	6

Table 1 continued

Nest	Date of parasite attack	Date filmed	Minimum no. of days parasite present before filming	Time between parasite removal and 2nd day's filming (days)	No. of host foundresses	At time of filming			
						No. of cells	No. of eggs	No. of Larvae	No. of worker
s16	19/04/2005	04/05/2005	15	1	5	81	3	25	1
s11	11/04/2005	04/05/2005	23	1	7	93	17	23	1
170	17/04/2005	10/05/2005	23	1	3	73	18	15	1
165	16/04/2005	17/05/2005	31	1	8	107	32	17	3
62	19/04/2005	20/05/2005	31	1	5	155	5	41	4
28	06/04/2005	04/05/2005	28	1	3	107	12	25	6
172N	22/04/2005	08/05/2005	16	2	2	73	17	15	4

Table 5-1: Details of the seventeen parasitised nests studied in this chapter.

5.5.a Initial preparation

Nests were identified and marked in the Farm field site in 2004 and 2005 (see Methods). A total of seventeen parasitised nests were filmed for 180 minutes both prior to and after parasite removal (Table 5-1). A total of seventeen matched unparasitised nests was also filmed before and after dominant removal, the dominant individual was determined as described in the General Methods chapter. The day before filming, individuals were marked using enamel paints (see methods). Nests were then filmed between the hours of 10 a.m. – 5 p.m. on clear, sunny days, to ensure normal wasp activity was recorded. On the evening (10pm onwards) of the first day of filming, the parasite (or host dominant on control nests) was removed from the nest. The nests were then filmed the next clear, sunny day, in order to record host behaviour without presence of the parasite. On the evening of the second day's filming, the nest was censused to check all members were present, and collected. Nests were then placed in a 4°C refrigerator for a maximum of 4 days before being stored at -80°C at Cadiz University. The nests and their associated wasps were separated in the 4°C refrigerator to ensure no brood were destroyed by wasp activity. Samples were stored at Cadiz for 1-2 months. At the end of the field season, samples were transported on dry ice back to U.C.L. and stored immediately at -80°C until being used for molecular analysis.

5.5.b Weighing Samples

After being stored in a -80°C freezer for 3-4 months at U.C.L., samples were weighed on a Sartorius balance, accurate to ± 0.001g. Samples were weighed immediately after removal from -80°C storage, to minimise weight loss through evaporation.

5.5.c Microsatellite Analysis

In order to determine maternity of brood on parasitised nests, I compared the genotypes of each offspring with those of potential mothers (adult females on the nest) using five microsatellite loci. This study represents the first time microsatellite markers have been used in *P. semenowi*.

In order to obtain genotypes, the samples underwent the following steps:

- **Sample preparation.**
- **DNA extraction and purification.**
- **PCR amplification of specific microsatellite loci.**
- **Determination of PCR product sizes.**

Detailed protocols for each of these steps can be found in Chapter 2 and Appendix 3. The five loci used in this chapter are now described.

5.5.c.i. Locus Pdom7

The original study which designed primers for this locus in *P. dominulus* indicated an PCR product of 160 base pairs (b.p.) in size (Henshaw 2000). Based on previous studies in the U.C.L laboratory, a size range of between 151-175 b.p. (7 alleles) was expected in *P. dominulus* (Cant et al. 2006). In my study the range was 142-184 b.p. for *P. dominulus* and 154-184 b.p. for *P. semenowi* (Figure 5:1).

The alleles generally appeared as a couplet of peaks. The upper peak, which showed greater intensity, was scored. The parasite allele distribution is similar to that of the host (Figure 5:1):

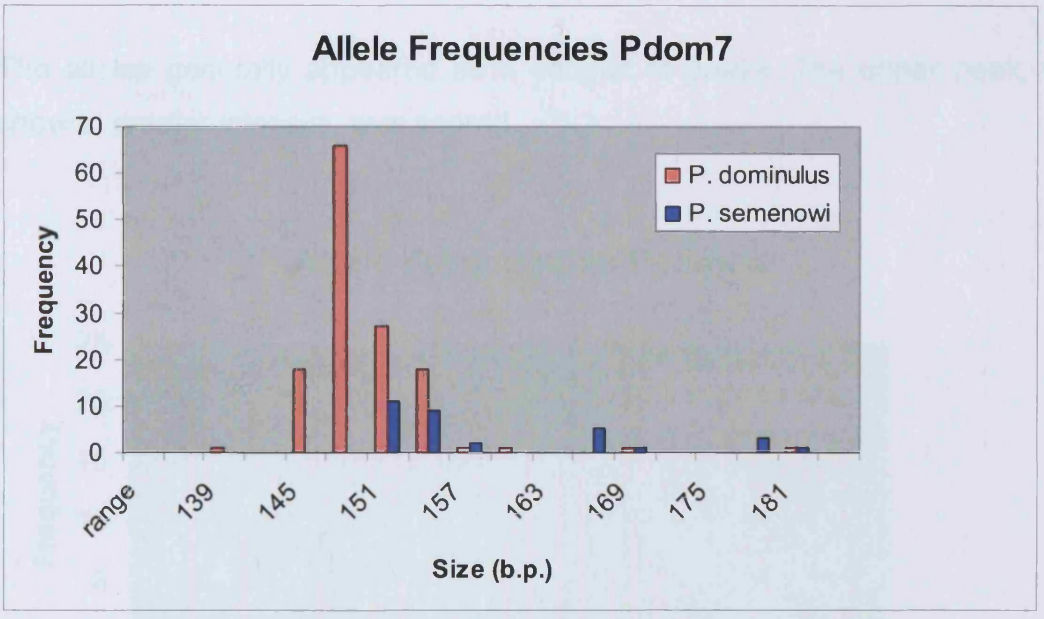

Figure 5:1: Relative Allele Frequencies of Locus Pdom7

5.5.c.ii. Locus Pdom20

This locus proved the most difficult to amplify. Often, samples had to be rerun to obtain products. The product typically consisted of a pair of peaks. Pdom20 products were originally placed around 236 b.p. (Henshaw 2000). In my study the range was 207-285 b.p. for *P. dominulus* and 246-276 b.p. for *P. semenowi* (Figure 5:2)

P. semenowi in this population has a narrower allelic size range at the upper end of the *P. dominulus* allelic range for Pdom20 (Figure 5:2). The sample size of parasites was smaller (15 parasites), suggesting that this could have been an effect of sampling. However, the distribution of alleles for both parasite and host in the upper size range is similar.

The alleles generally appeared as a couplet of peaks. The upper peak, which showed greater intensity, was scored.

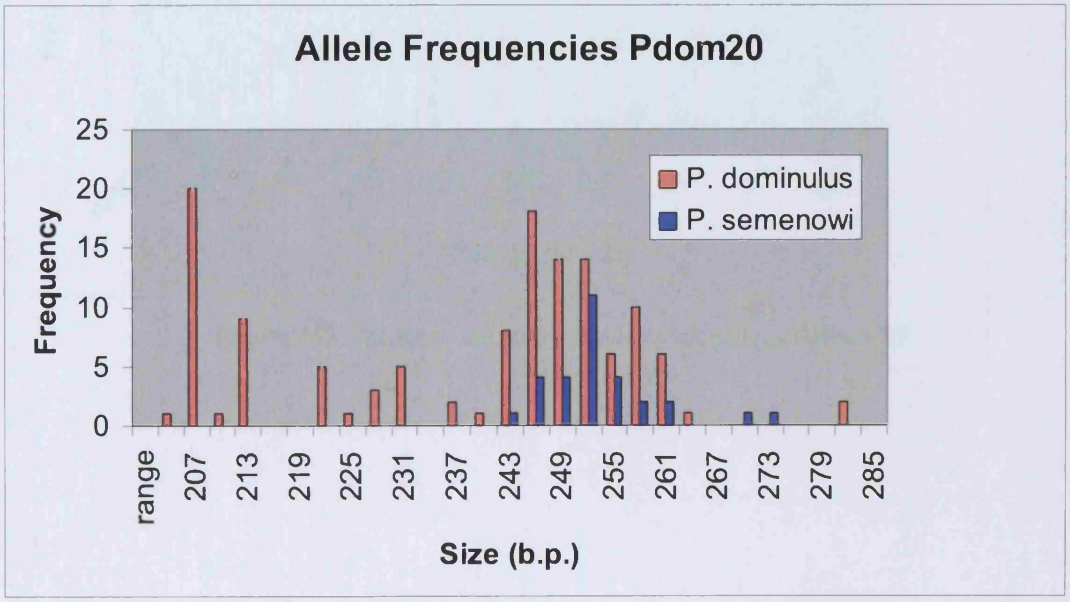

Figure 5:2: Relative Allele Frequency of Locus Pdom20.

5.5.c.iii. Locus Pdom127b

The original study which designed primers for this locus in *P. dominulus* indicated a PCR product of 119 b.p. size (Henshaw 2000). Based on previous studies in the U.C.L: laboratory, a size range of between 110-152 b.p. (15 alleles) was expected in *P. dominulus* (Cant et al. 2006). In my study the range was 105-153 b.p. for *P. dominulus* and 102-153 b.p. for *P. semenowi* (Figure 5:3).

The alleles generally appeared as a couplet of peaks. The upper peak, which showed greater intensity, was scored. The parasite allele distribution is similar to that of the host (Figure 5:3):

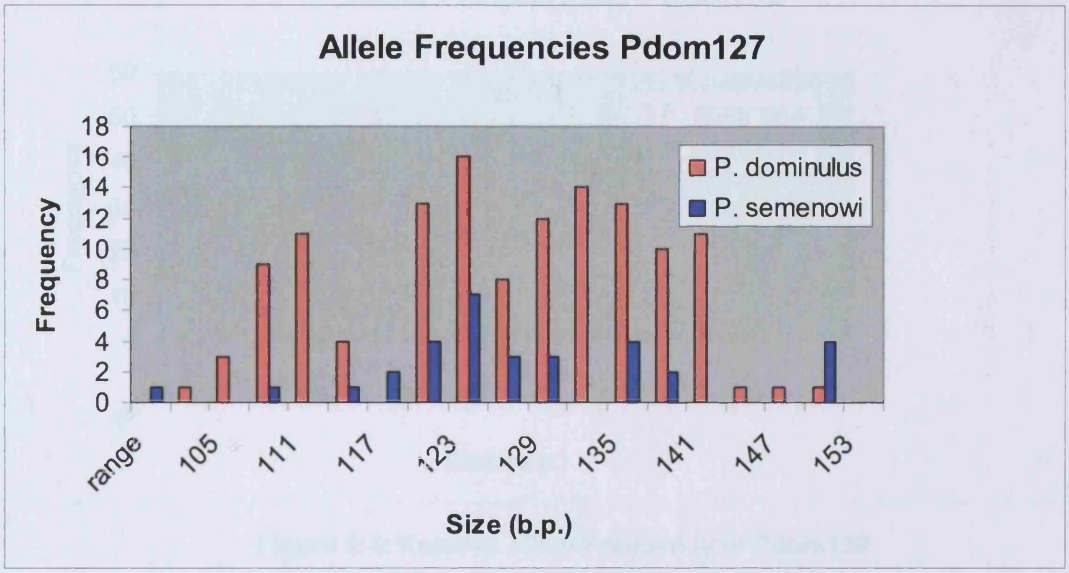

Figure 5:3: Relative Allele Frequency of Locus Pdom127

5.5.c.iv. Locus Pdom139

The original authors gave an example allelic size of 186 b.p. (Henshaw 2000). Studies based at U.C.L. gave a range of 177-213 b.p. over 12 alleles(Cant et al. 2006). In my study the range was 174-219 b.p. for *P. dominulus* and 186-231 b.p. for *P. semenowi* (Figure 5:4)

Whereas the host is distributed centred mainly around 192 b.p., the parasite seems to have a bimodal distribution centred around 192 b.p. (similar to the host) and 213b.p. (Figure 5:4).

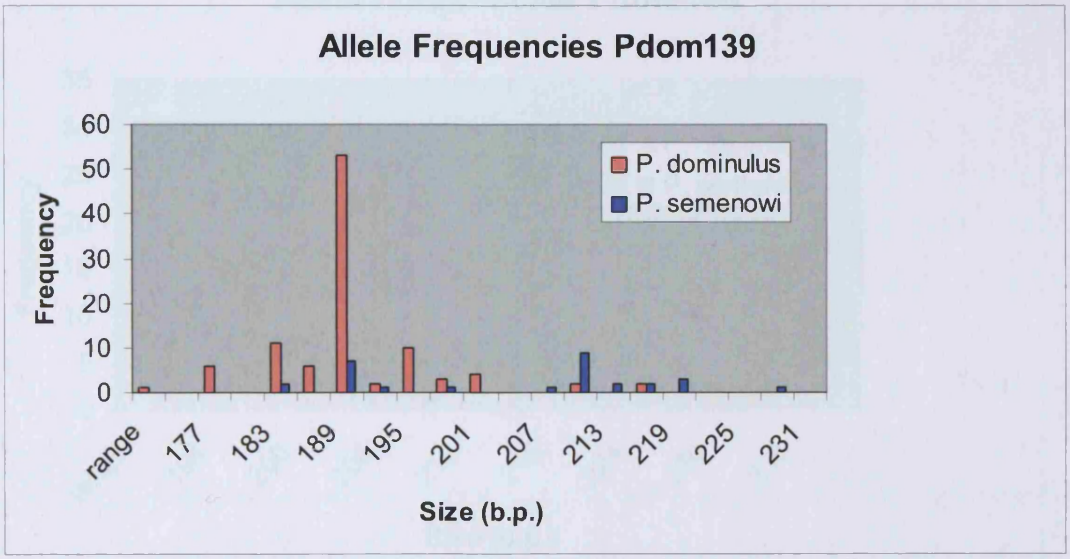

Figure 5:4: Relative Allele Frequency of Pdom139

5.5.c.v. Locus Pdom140

Previous studies gave a P.C.R. product size of 192 b.p. (Henshaw 2000). Previous studies at U.C.L. gave a range of between 195-237 b.p. consisting of 10 alleles (Cant et al. 2006). In my study the range was 194-236 b.p. for *P. dominulus* and 197-239 b.p. for *P. semenowi* (Figure 5:5).

Whereas the host is distributed centred mainly around 206 b.p., the parasite has a shifted allelic range towards 218 b.p. (Figure 5:5).

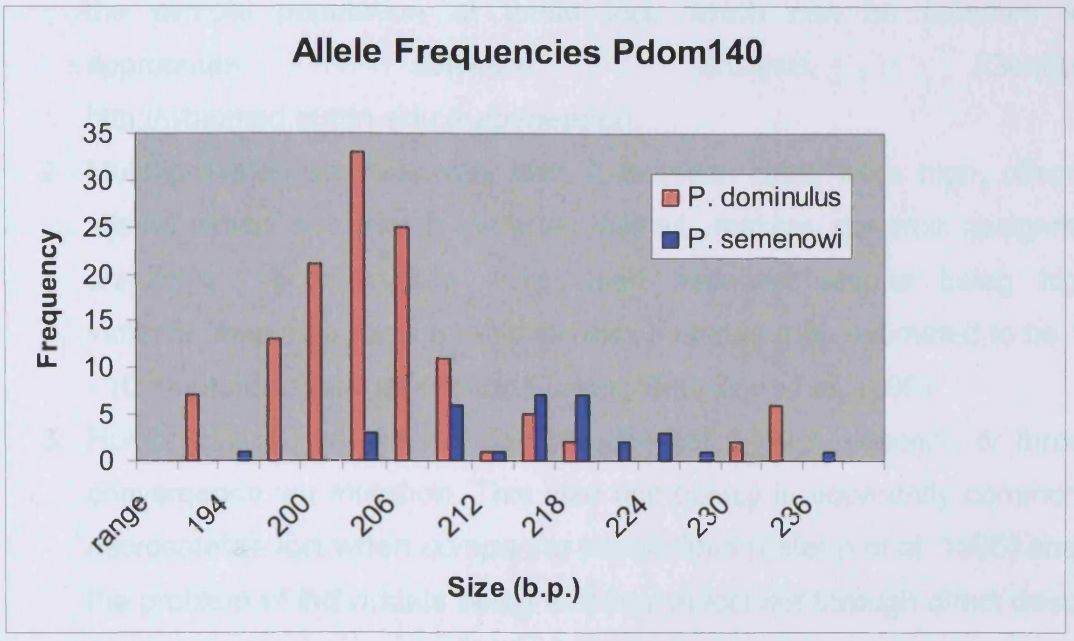

Figure 5:5: Relative Allele Frequency of Locus Pdom140

5.5.d Assigning Maternity

The use of microsatellite markers to determine maternity of host and parasite offspring depends on satisfying several criteria (Casteele et al. 2001);

1. Null alleles are rare or absent; null alleles would give a heterozygote genotype the appearance of a homozygous genotype (Brookfield 1996). Null alleles occur commonly when a mutation in the primer-binding site blocks primer binding and hence the amplification of the allele at that loci. If null alleles are common, then there will be a "heterozygote deficiency" in the sample population at those loci, which can be detected with appropriate software analysis (Genepop; http://wbiomed.curtin.edu.au/genepop/).
2. Mutation rates are relatively low; if mutation rates were high, offspring alleles would not match parental alleles, making parental assignment unreliable. Microsatellites were used because despite being highly variable, they also have a relatively low mutation rate, estimated to be 10^{-2} - 10^{-5} mutations per generation (Baker 2000; Zhu et al. 2000).
3. Homoplasy is rare. Alleles can be identical through descent, or through convergence via mutation. This size homoplasy is apparently common at microsatellite loci when *comparing* populations (Estoup et al. 1995) and so the problem of individuals being identical at loci not through direct descent from a common recent ancestor can possibly confound determination of maternity (Queller and Goodnight 1989). However, levels of homoplasy are thought to be small *within* a population (Schlotterer 1998). The likelihood of both individual parasites and hosts having identical alleles at all 5 loci is extremely low.
4. The genotyping is accurate; the use of microsatellites with three-base-pair repeat motifs makes distinguishing between alleles relatively easy. Multiple rescoring of alleles and the use of an identical internal size standard for each sample run adds to the accuracy.

An offspring could be assigned as either parasite or host if it shared at least one of its alleles at each locus with the parasite or a host foundress from its nest.

Also, as multiple mating is rare in *P. dominulus* (Queller et al. 2000) and probably *P. semenowi*, female offspring from the same mother should share a paternally-derived allele at each locus.

5.5.e Brood Mapping

In the field, brood were assigned to three distinct larval stages; stage 3 larvae were the largest stage before pupation, filling the cell and with pigmented mouth parts. Stage 2 larvae were smaller than stage 3, without pigmentation, but still filling greater than 2/3 of the width of the cell. Stage 1 larvae consisted of any larvae smaller than stage 2, usually filling less than half the width of the cell. These stage 1 larvae therefore consisted of all small larval stages.

In statistical analysis of feeding, because stage 1 and 2 larvae were not morphologically distinct other than in average size, larvae were categorised as either "small" (stages 1 and 2) and "large" (stage 3) larvae.

5.5.f Video Analysis

The video recordings provide a record of adult wasp behaviour on the nest. In order to examine specific behavioural interactions, analysis of these recordings had to be focussed and specific methodologies employed. Two main behaviours were studied:

- Feeding
- Brood removal

5.5.g Feeding and Brood Care

Adult wasps interact with brood in several different ways; checking cells for brood, feeding solid food to them, fanning them with wings and providing water and nectar. Analysis of feeding therefore had to focus specifically on behaviour related to actual feeding and not other activities. Two forms of brood care were recorded;

1. Feeding
2. "Cell Checking"

The former was defined as when an adult wasp with food present in its mouth placed its head into a cell for more than 2 seconds. The minimum time limit of 2 seconds served to differentiate feeding behaviour from simple cell contents checking, which usually lasts less than 1.5 seconds (see Appendix 2). The latter encompasses a wider range of behaviours, involving any behaviour where an adult wasp placed its head into a cell without holding food.

Feeding behaviour was recorded before and after parasite removal. As I am only interested host foundress feeding, any feeding by the parasite is excluded from the analyses. Overall feeding rates refer to total feeding per larva recorded over 4.5 hours filming.

Figure 5:6: A photograph of the video analysis equipment. A transparent acetate sheet placed over the screen was used to map the cells whilst still allowing a clear view of wasp activity.

Transparent, markable acetate sheets, placed over the video screen allowed nest cells to be mapped and numbered (Figure 5:6). Feeding directed towards each cell was recorded on the acetate sheet. The identity of the adult feeder was also noted, using their unique paint dot marking.

Acetate maps of feeding were overlaid on the brood maps made during the extraction of brood from the nests. Because nests were collected immediately after filming and wasps separated from their associated nests, the brood composition encountered in the laboratory is unchanged from when videoing in the field. Combining the results of molecular analysis and feeding observations therefore allowed determination of which adult feeds which individual and whether they are related.

5.5.h Brood removal

4.5 hours of video footage, recorded between 1-2 days (Table 5-1) after parasite removal, was observed for each of 19 parasitised nests (85.5 hours total footage). Brood destruction was judged to occur when a host foundress or worker entered a cell without any solid food in its mouth and emerged with larvae. Normally, this removal is conspicuous, with most or the entire larva being removed at once, making identification of this behaviour easy (personal observation). Removal of very small brood and eggs is probably not detectable in this way as they can be fully swallowed before the adult wasps head comes out from the cell, and hence could be hidden from view.

5.5.i Statistical Analysis – GLMM

For analysis of feeding rates received by individual larvae, a general linear mixed model with normal errors ("REML") was used in the Genstat 8.0 software package. Nest identity was fitted as a random effect in all models. Year of study, larval species/ whether the nest was parasitised (coded "A" = Host larvae on unparasitised nests, "B" = Host larvae on parasitised nests, "C" = Parasite larvae), larval size ("1" = small, "2" = large), group size, nest size (number of cells), and the relative day in the season were fitted as fixed effects. Fixed effect were dropped from the full model via backward elimination, until removal of any terms remaining led to a significant decrease in the explanatory power of the model ($p<0.05$). This was assessed using a Wald statistic that is asymptotically distributed as χ^2 (Genstat 8.0). Each significant term was separately added to the minimally adequate model to assess its significance. Relevant two-way interactions were also tested in the presence of main effects in this way, but were not included in the results unless significant.

5.6 Results

5.6.a **Feeding Of Larvae By Host Foundresses**

Only ten out of seventeen nests contained both parasite and host brood (Table 5-6, page 139). Nest s11 was not included in this analysis as video recordings were not of high enough quality to accurately record feeding. The numbers of known parasite and host larvae that feeding behaviour was recorded for is shown in Table 5-2:

Larval Stage	Host (Parasitised Nests)	Host (Unparasitised Nests)	Parasite
1 (small)	17	94	71
2 (large)	18	64	29

Table 5-2: Total numbers of parasite or host assigned larvae recorded being fed in feeding analysis experiments

5.6.b Do hosts preferentially feed their own larvae before parasite removal?

Before removal of the parasite or host dominant, parasite larvae were fed significantly less than host larvae (see Figure 5:7, GLMM, effect of species-nest, Wald = 3.55, p=0.029). There was also a significant interaction: small and large parasite larvae were fed consistently less than hosts, but large host larvae from parasitised nests were fed as much as hosts from control nests, whereas small host larvae were fed less than small control larvae (see Figure 5:7, GLMM, effect of species-nest x larval size interaction, Wald = 16.34, p<0.001).

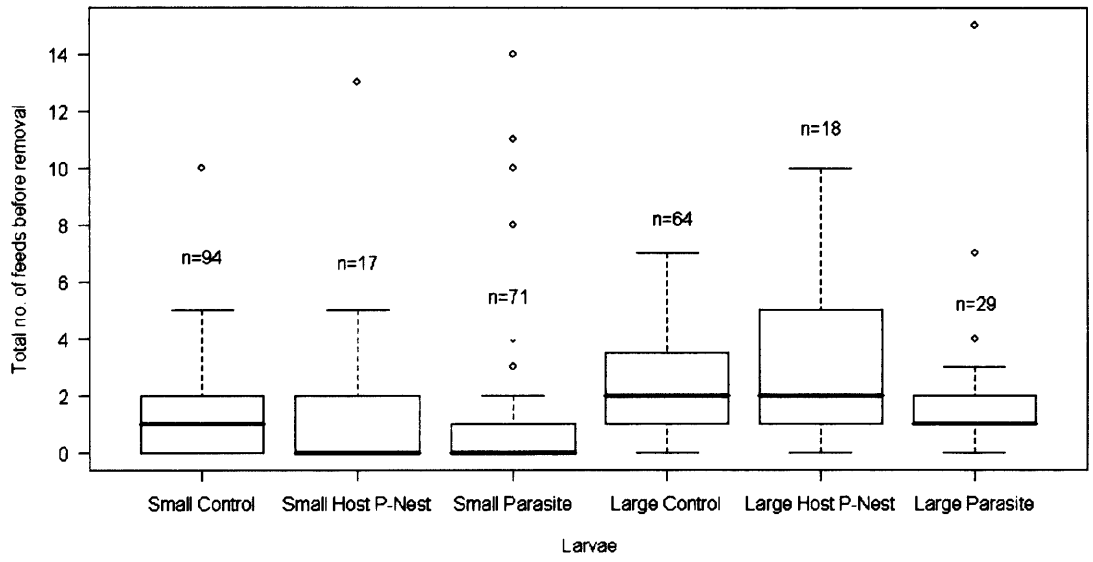

Figure 5:7: Total feeding before dominant/parasite removal, for different larval sizes (small or large). Nests are coded as follows: "Control" = *P. dominulus* larvae on unparasitised nests, "Host, P-Nest" = *P. dominulus* larvae on *P. semenowi* parasitised nests, "Parasite" = *P. semenowi* larvae. Bars indicate the 95% confidence intervals.

The minimal adequate GLMM model with the *overall feeding rate before removal of the parasite* as the response variable and nest identity as a random effect, revealed several significant effects (shown in bold in Table 5-3):

Model Terms	Wald Statistic	d.f.	p
Larval size	**34.18**	**1**	**<0.001**
Species + nest status of larvae	**3.55**	**2**	**0.029**
Nest Size (cells)	0.88	1	0.347
Group Size	0.09	1	0.766
Year	0.03	1	0.853
Relative day nest was filmed	0.03	1	0.862
Larval size x Species + Nest status	**16.34**	**2**	**<0.001**
Larval size x Nest Size (Cells)	**28.74**	**2**	**<0.001**
Larval size x Group Size	**31.6**	**2**	**<0.001**
Relative day nest was filmed x Year	**6.46**	**2**	**0.039**

Table 5-3: General Linear Mixed Model of factors affecting total feeding rates before parasite/dominant removal (n=293 larvae from 18 nests).

Other significant effects were either concerned with the species or size of the larvae, nest size, or with the time in season, or year, of filming.

Larger, more developed larvae were fed more than small larvae (see Figure 5:8, GLMM, effect of larval size, Wald = 34.18, $p<0.001$). Large larvae were fed disproportionately more as group size increased (GLMM, effect of larval size x group size, Wald = 31.6, $p<0.001$). Smaller larvae were fed less as nest size (cells) increased, but there was no significant change in feeding of large larvae as nest size increased (GLMM, effect of larval size x nest size, Wald = 28.74, $p<0.001$).

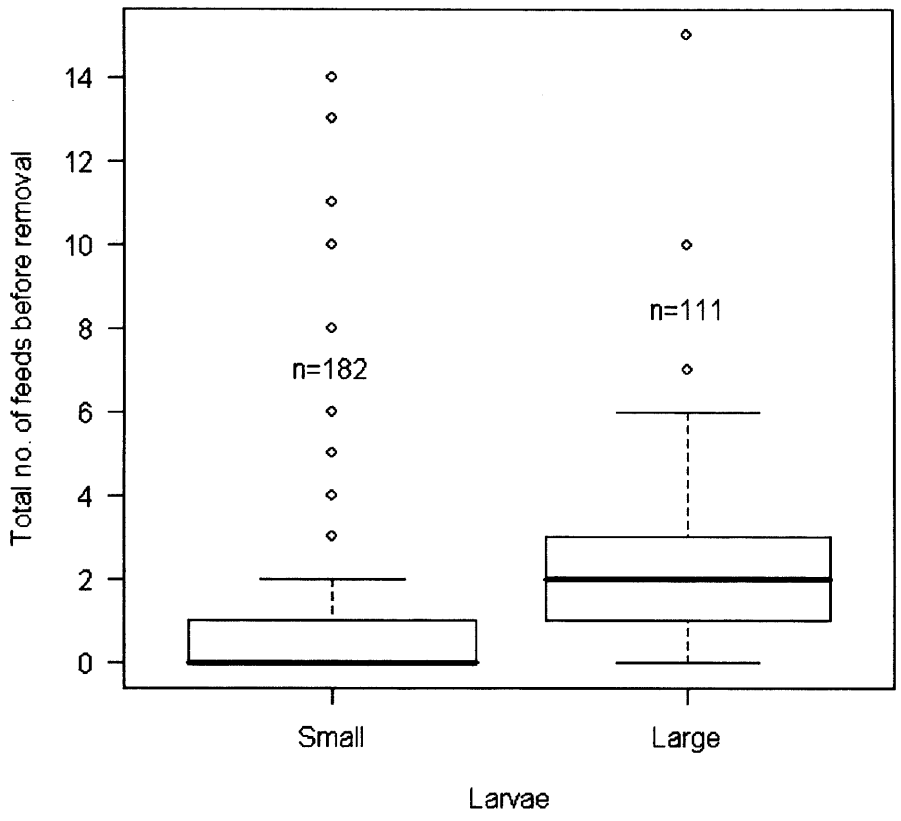

Figure 5:8: Total feeding before removal for all small and large larvae. Bars indicate the 95% confidence intervals.

Nests from 2005 had a lower feeding rate as the season progressed compared to 2004 nests (GLMM, effect of Year x relative day nest was filmed, Wald = 6.46, p=0.039). All other terms and two-way interactions did not have significant effects (p>0.05).

5.6.c Do hosts preferentially feed their own offspring after parasite removal?

After parasite removal, there was no significant difference in feeding of parasite larvae compared to host larvae on parasitised and unparasitised nests (see Figure 5:9, GLMM, effect of species and nest status of larva, Wald = 4.43, p=0.109).

The maximal GLMM model also included the overall feeding rate before removal as an additional fixed effect to those stated on page 130, in order to compare feeding rates before and after removal. Main terms and significant interactions are shown below (Table 5-4):

Model Term	*Wald Statistic*	*d.f.*	*p*
Feeding before	**22.94**	**1**	**<0.001**
Larval size	**47.75**	**1**	**<0.001**
Species + nest status of larva	4.43	2	0.109
Relative day nest was filmed	1.32	1	0.25
Year	0.55	1	0.459
Nest Size (Cells)	0.37	1	0.543
Group Size	0.01	1	0.93
Number of days of parasite presence	0.01	1	0.943
Larval size x Relative Day Filmed	**13.99**	**2**	**<0.001**
Larval size x Year	**9.47**	**2**	**0.009**

Table 5-4: General Linear Mixed Model of factors affecting total feeding rates after parasite/dominant removal (n=293 larvae from 18 nests).

Feeding levels did not differ significantly before and after removal: larvae that were fed relatively more before removal were still fed relatively more afterwards (GLMM effect of feeding before removal; Wald statistic = 22.94, $p < 0.001$).

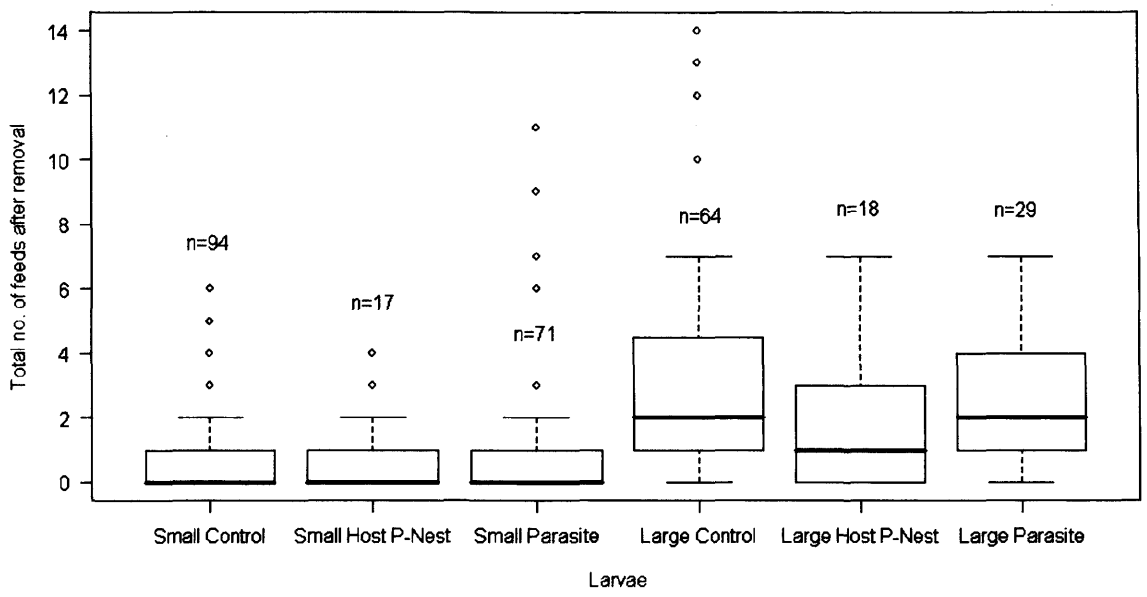

Figure 5:9: Feeding rates of parasite and host larvae after removal of the dominant individual (P = Parasitised Nests). Bars indicate the 95% confidence intervals.

Large larvae were fed significantly more than small larvae after removal (Figure 5:9, GLMM, effect of larval size, Wald = 47.75 $p<0.001$). There were two significant interaction terms. Larger larvae were fed more after removal as the season progressed (GLMM, effect larval size x Relative day filmed interaction Wald = 13.99, $p=0.001$). Larger larvae were fed more after removal in 2004 than 2005 (GLMM, effect of larval size x Year interaction, Wald = 9.47, $p = 0.009$).

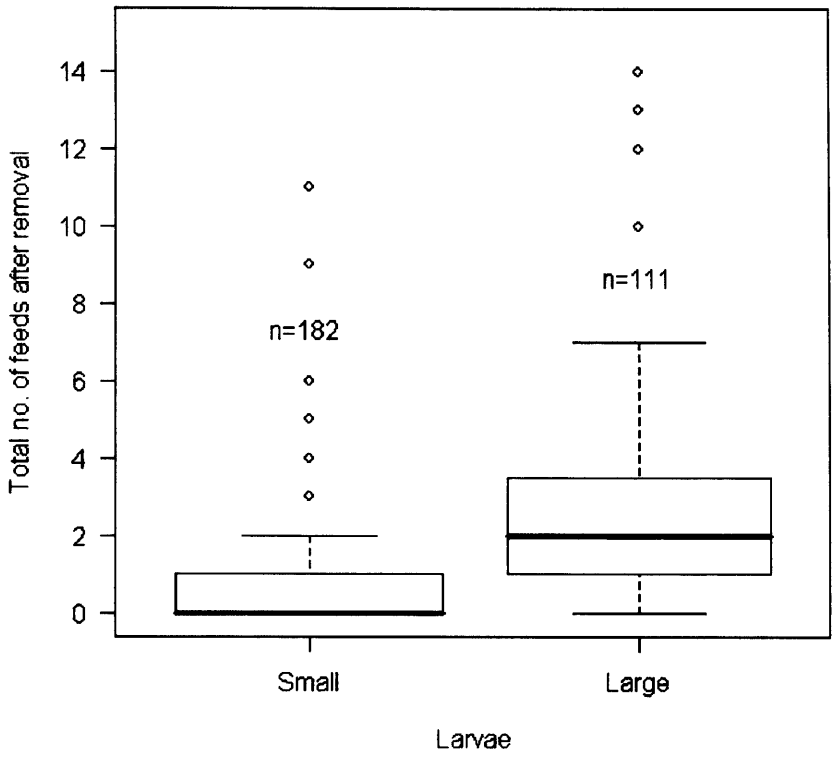

Figure 5:10: Total feeding before removal for all small and large larvae

5.6.d Do hosts remove parasite brood after short or long term parasite removal?

Brood removal by hosts was an extremely rare event on the day after removal of the parasite. Microsatellite analysis indicates that parasite brood was present on all 17 parasitised nests analysed. 4.5 hours of video recordings were observed for each of the 17 nests after removal of the parasite. Only one instance of brood removal was recorded from video analysis, by a host worker: the removal of a pupa (Nest 59, 2004).This suggests that for short term absences of the parasite, hosts do not seize the chance to remove large parasite brood.

A pilot study of 6 parasitised nest was undertaken to determine whether host foundresses removed brood after long term absence of the parasite. Parasites were removed on the evening of 24/04/05 and nests mapped for brood. Brood were then mapped at weekly intervals for 2 further weeks. Any removal of brood could therefore be determined (Table 5-5):

Nest	Nest Size	No. of larvae at start	Brood removed	
			Week 1	Week 2
R23	85	31	1 small larva	1 small larva
R21	66	21	None	None
R37	131	31	None	1 small larva
R28	89	34	None	None
R31	94	26	None	None
R45	102	38	2 small larva	None

Table 5-5: Recorded brood removal after removal of the parasite after 1 and 2 weeks absence.

Between 54-100% (mean 83%, n= 17 nests) of brood analysed in this thesis was of parasite origin. The maximum amount of brood removal observed in this experiment was 6% of total brood, so removal as a strategy of ridding the nest of parasite brood does not seem to be performed by *P. dominulus* foundresses, even after prolonged *P. semenowi* absence. The pilot was not expanded as other groups have reported a similar result and parasitised nests were needed for other studies (R. Cervo, personal communication). It is unknown whether the rate of removal recorded here differs from unparasitised nests. Also, the fate of "removed" brood was not certain; predation could have also caused disappearance of brood. Therefore, no firm conclusions about brood removal behaviour can be made, other than that it does not seem to happen at a high rate on previously parasitised nests.

5.6.e Host Reproduction on Parasitised Nests

Host reproduction is not totally inhibited on parasitised nests. Ten out of seventeen parasitised nests studied contained host brood, amongst the larvae selected for microsatellite analysis. Details of the nests that did contain host brood are given in Table 5-6:

Nest	Year	No. Parasite Larvae	No. Host Larvae	Host Larvae (Proportion)	Host Larvae Laid After Parasite Arrival	
					No.	Proportion of total larvae
nr121a	2004	6	4	0.40	-	-
s172	2005	5	1	0.17	-	-
170	2005	7	6	0.46	-	-
165	2005	14	1	0.07	-	-
172N	2005	11	3	0.21	-	-
nrP4	2004	15	5	0.25	3	0.15
16	2004	21	6	0.22	2	0.07
s16	2005	13	9	0.41	7	0.32
s11	2005	12	8	0.40	1	0.05
62	2005	10	2	0.17	1	0.08

Table 5-6: The number of host and parasite larvae in parasitised nests. Green highlighting indicates nests where host larvae are younger than the oldest parasite larvae. The final columns give the number and proportion of the total larvae of these younger host larvae.

Five out of ten nests contain host brood that were of a lower developmental stage (younger) than the oldest parasite brood. The host foundresses on these nests were therefore likely to have laid eggs which were allowed to develop to a larval state during parasite occupation. There is no significant difference in the absolute number or proportion of parasite or host larvae in nests that did have host brood produced after parasite attack compared with nests that did not (Welch Two Sample t-test, d.f. = 7.5, $p > 0.05$).

5.7 Discussion

5.7.a Differential feeding by hosts

The main aim of this chapter was to test whether host foundresses preferentially feed their own offspring or whether the parasite or its offspring have developed a countermeasure to obtain more food. Analysis of host feeding on parasitised nests prior to parasite removal suggests that host foundresses feed their own large larvae in preference to the large parasite larvae (Figure 5:7). I hypothesised this behaviour would occur as hosts gain no benefit from feeding unrelated parasite offspring and so should focus their effort towards related brood. It therefore appears that *P. semenowi*, despite its successful adaptations to enter and usurp *P. dominulus* nests, has not developed methods of monopolising host foundress feeding in the time immediately after usurpation. There are several possible reasons why *P. semenowi* may have not yet developed adaptations to exploit feeding by host foundresses.

One explanation is that female host larvae are likely to become workers when they mature, as the parasite attacks just prior to the production of the first workers (Demolin and Martin 1980; Reeve 1991; Zacchi *et al.* 1996). Host workers will help forage for food and rear parasite young, so are beneficial to the parasite. By having more host workers, the parasite gains extra productivity on the nest and can therefore raise more of its own young. Rearing parasite offspring also benefits the parasite as it gains direct fitness benefits through doing so. Parasite adults might therefore seek to ensure that all larvae are fed, as they receive benefits through either host or parasite larvae being fed.

Host foundresses, however, would be expected to preferentially feed their own offspring, as they gain no benefit from feeding the parasite larvae. Host foundresses would benefit most from rearing their own young if they were destined to become reproductives, since workers rarely reproduce unless all foundresses leave the nest (Nonacs 2002; Strassmann et al. 2003). Workers on nests parasitised by *P. semenowi* and *P. sulcifer* have been observed to have developed ovaries and lay eggs, so rearing workers is possibly not entirely futile for *P. dominulus* foundresses (Turillazzi *et al.* 1991). Larval nutrition has been implicated in caste determination in *Polistes*, with well nourished larvae becoming reproductive gynes and less well nourished larvae becoming workers (Wheeler

1986; O'Donnell 1998). Hosts would therefore benefit two-fold in preferentially feeding their offspring; they would hasten their development and possibly switch their caste fate to become reproductives.

The feeding behaviour of host workers might be more important for the parasite than the feeding behaviour of host foundresses. The workers comprise the vast majority of the workforce on nests during most of the period following parasite attack (Reeve 1991). The parasite may therefore have evolved adaptations to exploit workers rather than foundresses. I did not study *P. dominulus* worker feeding behaviour, but a study of workers on nests parasitised by another *Polistes* parasite, *P. sulcifer*, found that parasite larvae were fed preferentially by such workers (Cervo et al. 2004, discussed further on p. 142). Host workers, hatching after parasite attack, do not receive an obvious cue indicating that they are being parasitised: the initial violent usurpation. If they learn nestmate recognition cues from the nest *upon emergence*, then they may not be able to discriminate between host and parasite larvae (Gamboa *et al.* 1986). In summary the parasite might tolerate host foundresses preferentially feeding their own offspring, because they will help rear more host workers, which might not show the same feeding bias as foundresses.

The above findings pose a further question: why do *P. semenowi* offspring not themselves monopolise feeding, as occurs in *P. sulcifer* and many avian brood parasites systems (Kilner et al. 1999; Davies 2000; Cervo et al. 2004)? Adult *P. semenowi* parasites might be relatively indifferent to host feeding preferences, but parasite larvae might be expected to be selfish and evolve mechanisms independent of adult adaptations, to monopolise feeding (Nonacs and Tobin 1992). Parasite larval selfishness should be more pronounced than host larval selfishness, as parasite larvae are always reared on nests containing unrelated (host) brood, whereas host larvae will generally be reared with relatives, as the majority of nests remain unparasitised by *P. semenowi* (see Chapter 1). Selfishness on the part of hosts could damage the development of relatives and hence reduce the indirect fitness benefits they gain from relatives being reared. The selfishness of *P. semenowi* larvae should be pronounced considering the high risk of mortality due to group failure in my population and the later time in the season in which they attack (Shreeves *et al.* 2003). My study, however, does not

support the hypothesis that parasite brood have developed ways of monopolising host feeding, as there was no bias in feeding towards parasite larvae.

5.7.b Feeding by host workers on nests attacked by *Polistes* social parasites

This study contrasts with the findings of a previous study where *P. sulcifer* larvae (another *Polistes* social parasite) were fed more than larvae of their host *P. dominulus* (Cervo et al. 2004). It remains possible that *P. sulcifer* larvae have evolved means to elicit more feeding whereas *P. semenowi* larvae have yet to do so. A comparison of larval behaviour of the two parasite species may reveal such differences.

There are several other possible reasons why my findings differed from the previous study. Cervo et al. (2004) did not control for larval developmental stage, but compared the average attention directed towards all stages of host versus parasite larvae. Parasite larvae may have been fed more, but this may have simply been a by-product of there being more large parasite larvae than host larvae. In my study, more feeding was directed towards more developed larval stages, irrespective of whether they were parasite or host, both before and after parasite removal (p. 132-135). This could explain the apparent feeding bias towards parasite larvae in Cervo et al.'s study. However, a reanalysis of my data without larval stage as an explanatory variable did not show a bias in feeding towards parasite larvae (E. Almond, unpublished). Another possible explanation for Cervo et al.'s findings was that food was given *ad libitum*, so host workers could feed all larvae at the maximum rate they could take up food. If parasite larvae had a greater ability to take up food, for example if they were larger, then they would be observed to be fed more simply because they *could* be fed more.

Another difference between Cervo et al.'s study and mine is that Cervo et al. looked at feeding by host workers which may not be able to determine whether the nest is parasitised (p. 140, Zacchi et al. 1996; Lorenzi et al. 2004). If workers were unable to discriminate parasite from host offspring, then the feeding patterns they exhibit might be different from host foundresses which could discriminate. Further study of worker feeding behaviour on nests parasitised by

P. semenowi could test the hypothesis that worker and foundress behaviours differ.

5.7.c Does parasite presence affect host feeding patterns?

Before parasite removal, host foundresses fed related offspring more than parasite offspring (page 135). I hypothesised that parasite presence may be mediating a kind of "Mafia" effect, forcing the host foundresses into feeding parasite larvae (Zahavi 1979). If anything, parasite larvae appeared to be fed relatively *more* after parasite removal (Figure 5:9), arguing against such an effect. After parasite removal, there was no difference in feeding rates of parasite and host larvae, whereas before removal parasite larvae were fed less than host larvae (Figure 5:7).

The presence of the parasite was accompanied by host foundresses biasing their feeding towards host larvae. Removal of the parasite apparently stopped this bias. One possible explanation is that host foundresses responded to parasite presence, and once this cue was removed no longer biased their feeding (c.f. cuckoo presence and associated egg rejection response of reed warblers; Davies et al. 1996). However, because host foundresses can seemingly differentiate between host and parasite larvae, it is difficult to explain this change in behaviour. Removal of the parasite may have simply disrupted the activity of the nest and in some way lessened the level of differential feeding. If host larvae could detect that they were parasitised by *P. semenowi*, then they might be expected to behave more selfishly, which may have resulted in increased begging for food to compete with parasite larvae. Such begging may have stopped on removal of the cue.

Another possible explanation for the lack of feeding bias after removal is that my study was limited to only 9 parasitised nests and their controls. It may be that the non-detection of a feeding difference after removal was due to a lack of power in the statistical analysis. The length of time for which feeding behaviour was recorded may also have affected the power of my analyses. A larger sample size of parasitised nests, or longer observation of feeding, would have made the tests performed more powerful, but time constraints on filming and high levels of nest mortality in the field limited the number I could study in the season. The low

numbers of host brood on parasitised nests, in comparison with parasite brood, cannot be controlled for but would also have had an effect on statistical power.

5.7.d Host Reproduction on Parasitised Nests

Host reproduction after parasite usurpation still occurs, at least on some nests (Table 5-6). After at least fourteen days of parasite presence, ten out of seventeen parasitised nests contained host larvae. The remaining seven nests did not have every larva genotyped, so it is possible that hosts were present on them too. Nests which did contain host brood were collected 15 – 55 days after the parasite first attacked. Previous studies suggest that *P. dominulus* larvae, on nests parasitised by *P. sulcifer*, take on average 16.6 ± 0.8 days to develop to pupae from hatching (Cervo *et al.* 2004). In the majority of nests examined, therefore, any host brood laid prior to parasite attack should have pupated and so would not have been included in my analysis. The majority of host brood present therefore were probably from eggs laid after parasite arrival, in the presence of *P. semenowi*.

These data suggest that the parasite does not completely halt host reproduction, in agreement with findings in other *Polistes* social parasites (Mead 1991; Dapporto *et al.* 2004). If the parasite is "bribing" host foundresses to stay, the amount of reproduction ceded is seemingly small in the majority of cases. A further study examining how much reproduction subordinates obtain on unparasitised nests may give a clearer indication of whether the parasite bribes subordinates above the normal, unparasitised, level.

5.7.e Parasite Brood Removal by Host Foundresses

In both the short (<24 hours) and long term (2 weeks), removal of parasite brood, or brood in general following parasite removal, does not occur at a high level (maximum observed level = 6% of total brood over two weeks, see p.137). Conspecific brood destruction is well documented in *Polistes*. Usurpers have been observed to destroy the majority of young host brood, but still raise older host brood (Klahn 1988; Cervo and Turillazzi 1989). The natural rate of brood destruction in *P. dominulus* was not recorded in my long term pilot study, so it is

unknown whether removal rates exhibited by groups' parasitised by *P. semenowi* differed from removal rates of unparasitised groups during the same period. A large scale study with control unparasitised nests would allow any differences to be found.

There are several possible reasons why *P. dominulus* hosts do not destroy large numbers of parasite brood. Parasite mimicry of host brood might mean that hosts cannot accurately discriminate between host and parasite larvae. However, in *P. sulcifer*, parasite brood were found to have different epicuticular hydrocarbon signatures to host larvae (Dani *et al.* 2004). Whilst it seems host foundresses can discriminate enough to differentially feed their own offspring in preference to parasite larvae, parasite larvae were still fed, perhaps indicating that discrimination was not always accurate. If hosts lack the ability to always discriminate accurately, they would suffer the cost of destroying their own brood if they made recognition errors (Cervo 2006). Costs of making recognition mistakes and rejecting related brood have been best studied in avian brood parasite systems (Davies and Brooke 1988; Lotem et al. 1995). In one study of Reed Warblers parasitised by the Common Cuckoo, Reed Warblers made recognition errors 30% of the time when a mimetic cuckoo egg (real or model egg) was present in their clutch, ejected one of their own eggs rather than the cuckoo egg (Davies and Brooke 1988).

In the only study of differential oophagy in *Polistes biglumis*, eggs were transplanted from nests of non-relatives to experimental nests, foundresses destroyed 69% of eggs laid by a "foreign" queen, 25% of their own eggs that were experimentally manipulated (but not from a different nest) and 14% of eggs from their own nests that were not manipulated in any way (Lorenzi and Filippone 2000). The experimental manipulation undoubtedly caused an increase in overall oophagy, making it difficult to assess the true level of discrimination. The same study showed that on 4 nests, foundresses were able to discriminate their own eggs from those of the social parasite *P. atrimandibularis*, destroying an average of 60.4% of parasite eggs. Low sample sizes and the effect of manipulation again did not allow statistical assessment of true discrimination ability, but the level of parasite egg oophagy was greater than the rate of oophagy towards host eggs (25%).

Recognition of alien brood taken from other nests may be easier than discriminating alien brood laid on the home nest. *P. atrimandibularis* adults have

been shown to deposit parasite-specific epicuticular hydrocarbons upon the nest surface (Turillazzi et al. 2000). These chemicals have been implicated in nestmate recognition (p. 107). If these chemicals are distributed evenly, then both host and parasite larvae would be exposed to them. Likewise, if such compounds were added by the parasite to food prior to feeding, then all larvae fed would be exposed. Host discrimination of parasite brood on the basis of parasite-specific compounds would therefore be disrupted as host larvae would also bear parasite chemicals. Likewise, host-specific compounds present on the nest could be taken up by parasite larvae, further reducing host discrimination ability (Lorenzi *et al.* 2004).

Further experimentation is needed to discover whether the parasite adult does disrupt discrimination. One possible experiment in the laboratory would be to split an unparasitised nest into two halves. The parasite would then be allowed to attack one half, whilst the other would be left untouched by the parasite. Host wasps would have to be separated evenly between the two halves. Brood or foundresses could then be transplanted from each nest half to the other and brood destruction or feeding behaviour observed. Foundresses from naïve (non parasitised) nest halves would be expected to destroy any transplanted larvae from parasitised halves, as these larvae would have parasite cues which would not have been learnt as nestmate recognition cues by the naïve hosts. If the parasite compound cannot be used to discriminate, no destruction would occur. A control group, of split nests where both halves are not parasitised, would allow determination of the effect of manipulation on brood destruction rates.

5.8 Summary

This chapter aimed to test whether the parasite manipulates host foundresses into caring for its young. This manipulation could take two forms; manipulation to cause acceptance and provision of care for parasite brood; and manipulation causing parasite brood to be favoured over host brood. My study is consistent with the first form of manipulation: parasite brood were not destroyed in great numbers before or after parasite removal. However, host foundress feeding bias was detected towards host brood in presence of the parasite adult. Parasite presence did not seem to ensure parasite larvae were fed as much as host larvae. Host foundresses, therefore, do not seem to be manipulated beyond accepting parasite brood, and seem to be able to differentially feed their own young.

Host foundresses do reproduce directly on nests parasitised by *P. semenowi*, but further research is needed to test whether these offspring develop into reproductives, as opposed to workers. The amount of reproduction ceded by the parasite in any case appears to be small.

Chapter 6. **Summary**

In this chapter I will briefly summarise my main findings in the context of the main aims stated in the introduction chapter: has *P. dominulus* developed counter-adaptations against *P. semenowi* and has *P. semenowi* itself developed counter-adaptations to retain and manipulate hosts after attack?

6.1 Abandonment

Immediately following the initial attack of *P. semenowi*, *P. dominulus* foundresses on 13.6% of parasitised nests permanently abandoned the nest in my population. The rate of abandonment observed was significantly higher than the average daily abandonment rate of nests not exposed to the parasite, but not significantly higher than the maximum daily abandonment rate observed for such unparasitised nests. Host foundresses, on parasitised nests that did not immediately abandon after the initial parasite attack, were no more likely to abandon than host foundresses on unparasitised nests. Abandonment therefore does not seem to be widely used as a host anti-parasite strategy and is mainly employed during the initial invasion.

P. semenowi adults attack large groups with large nests (in terms of number of cells). Foundresses on such nests were less likely to abandon before worker emergence than smaller nests. These findings suggest a possible *P. semenowi* strategy of selecting nests that are both more likely to help the parasite support more brood and more likely to survive long enough in the season to rear parasite brood, as found previously by Shreeves et al. (2003).

6.2 Aggression and effort

P. semenowi adults initiated less total aggression than host dominants on unparasitised nests, but the profile of individual aggressive behaviours they exhibited was largely similar to that exhibited by host dominants. Subordinate host foundresses on nests parasitised by *P. semenowi* were more likely to both

initiate and receive mounting behaviour than subordinates on unparasitised nests, but the parasite was not more likely to receive mounts from subordinates than host dominants, suggesting this mounting behaviour was concentrated mainly amongst subordinates. When the parasite was removed, subordinates on parasitised nests were less likely to receive mounts than before removal, whereas no significant change in subordinate mounting behaviour was observed on removal of a host dominant from unparasitised nests. Further study of aggression exhibited by different ranked subordinates on parasitised nests is necessary to determine whether the parasite's reduction in aggression is a specific strategy of controlling hosts or perhaps just a by-product of its greater fighting ability.

There was no evidence that host subordinates worked harder on parasitised nests than on unparasitised nests. Individuals on both parasitised and unparasitised nests that received higher levels of aggression spent less time off the nest, yet *P. semenowi* adults were less aggressive than host dominants. Because subordinates on unparasitised and parasitised nests did not differ in foraging effort, it is possible therefore that dominant individuals do not directly control subordinate effort. Further study of subordinate activity patterns after receiving parasite or dominant aggression would test this hypothesis further.

6.3 Host Reproduction and Differential Feeding

Host subordinates preferentially fed host larvae in preference to parasite larvae, but only before removal of the parasite. Removal of the parasite led to feeding rates directed towards parasite and host larvae not being significantly different. I hypothesised that host foundresses may be allowed to preferentially feed their own offspring as part of the parasite's long term strategy, as long as such offspring were destined to be workers. Soon after parasite invasion host workers comprise the majority of the host workforce and may therefore be more important in terms of the parasites potential reproduction. If the parasite adult and brood had adaptations to exploit workers, which possibly receive no cue that they are parasitised, then exploiting worker effort may be more important than exploiting foundresses. Further studies may determine if host worker feeding patterns differ from foundress feeding patterns on nests parasitised by *P. semenowi*.

Host foundresses are able to reproduce on parasitised nests, but the majority of offspring in the nest are the progeny of the parasite. Hosts therefore probably do not remain on parasitised nests simply because they receive reproductive concessions from the parasite, although comparison with subordinate reproduction on unparasitised nests may add weight to this argument.

Brood removal by host subordinates does not occur at a high rate two weeks after removal of the parasite. Parasite brood are therefore likely to have developed adaptations to be accepted by host foundresses, as suggested in studies of *P. sulcifer* (Cervo et al. 2004; Cervo 2006). Unlike *P. sulcifer* larvae, I found no evidence that *P. semenowi* larvae have developed adaptations to bias host feeding towards them.

6.4 Are host foundresses "deceived" by P. semenowi?

A more general aim of this thesis was to investigate whether host foundresses are fully deceived by the parasites nest infiltration strategy. My studies of host foundress responses to being parasitised by *P. semenowi* suggest that the hosts are not "deceived" beyond accepting parasite larvae rather than destroying them. Some host groups abandon the nest if the parasite attacks and those groups that stay feed their own offspring in preference to parasite offspring. The parasite adult does not seem to cause host subordinate foundresses to work harder than unparasitised host subordinates. Further studies are still needed to discover whether the parasite deceives host workers, whose contribution to parasite reproductive success may be more important than that of the foundresses.

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Chapter 8. **Appendix**

8.1 Appendix 1 - Average Weather Data for Conil 1971-2000

Month	Mean Temp (°C)	Mean Max. Daily Temp. (°C)	Mean Min. Daily Temp. (°C)	Mean No. Days Of Precipitation > 1mm	Mean Number of Clear Days
Jan	10.7	15.9	5.4	7	10
Feb	12	17.5	6.6	7	8
Mar	14	20.2	7.7	5	10
Apr	15.4	21.5	9.4	6	7
May	18.4	24.6	12.1	4	8
Jun	22	28.8	15.3	2	14
Jul	25.5	33	18	0	21
Aug	25.7	33.1	18.4	0	20
Sep	23.5	30.2	16.8	2	13
Oct	19.1	25	13.3	6	9
Nov	14.7	20.1	9.2	7	10
Dec	11.9	16.8	7.1	9	8

Table 8-1: Temperature ranges, mean number of days precipitation and number of clear days for the period 1971-2000 for Conil de la Fronterra, Spain.

8.2 Appendix 2- Determining Feeding From More General Care Behaviour

In order to analyse the provisioning behaviour of wasps on nests with parasite, both before and after parasite removal, the definition of what constitutes feeding behaviour had to first be derived. Observation of wasp activity on the nest left some ambiguity in whether a specific cell was being fed or merely "checked".

In order to analyse the behaviour filmed during the 2004 field season, the problem of what constitutes "feeding" still has to be faced. A reasonable assumption to be made is that feeding is most likely to occur when the adult wasp has food visible in its mouth and is visiting cells. From behavioural observation both in the field and from film, it is clear that food laden wasps have at least two types of behaviour, "checking" and "feeding". "Checking" is a behaviour whereby the adult wasp spends a short amount of time (0-1.5 seconds) over a nest cell, rarely placing its head into the cell. "Feeding" behaviour occurs when the adult wasp spends over 2 seconds over a cell, with its head and part of the thorax pushed into the cell. An analysis of time spent by a food laden adult wasp over (and in) individual cells give the following frequency distribution:

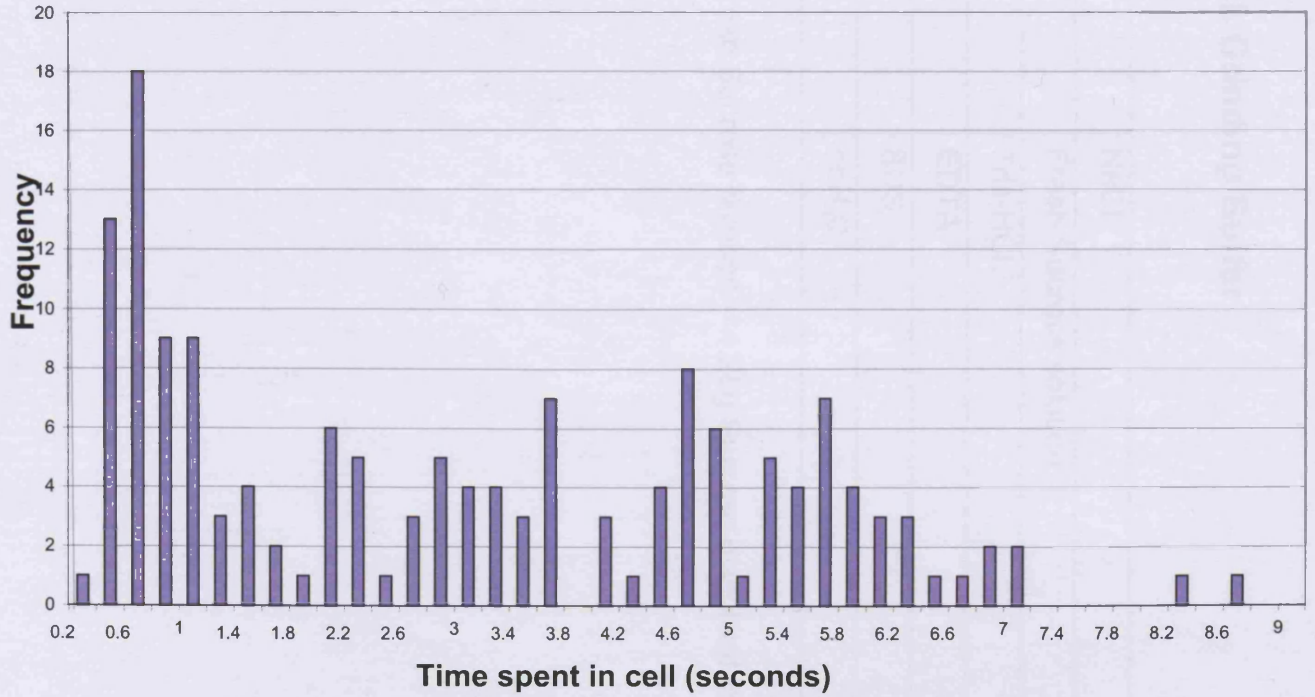

Figure 8.2-1: The frequency distribution of time spent in individual cells by a food laden adult wasp.

It is possible that in some of the "feeding" episodes no actual feeding occurred. Without direct field observation of each individual feeding incident, it would be impossible to be completely sure of food being passed from adult to larva. Since this observation is not possible using the film alone, any analysis using filmed observation will therefore make the assumption that feeding is highly likely to occur after a food laden wasps spends at least 2.0 seconds interacting with a cell.

8.3 Appendix 3 - DNA extraction, amplification and sequencing of loci

8.3.a Solutions used in DNA extraction:

8.3.a.i. Grinding Buffer

1M	NaCl	800µl
1M	Fresh Sucrose solution*	1600µl
1M	Tris-HCL	800µl
0.5M	EDTA	800µl
20%	SDS	20µl
	ddH_20	3980µl

*1M Fresh Sucrose solution = 4.28g Sucrose dissolved in 12.5 ml ddH_2O

8.3.a.ii. 10X TBE

dH_20	To make solution up to 1L
Tris	103g
Boric Acid	55g
EDTA	9.3g

8.3.a.iii. 1X TBE

10X TBE	100ml
dH_2O	900ml

8.3.a.iv. DNA dilution

The DNA extraction of adults and large brood needs to be diluted before use in the PCR. A 1:10 dilution was used. DNA extractions from small brood were not diluted in this way.

DNA extraction	2µl
ddH_20	18µl

8.3.b DNA Extraction for Microsatellite Analysis

1. Samples should be placed on ice, with no more than 20 samples selected at one time to limit damage to DNA due to thawing.
2. Label a tube for each sample and pipette into these **150 µl Grinding Buffer.**
3. Wash scapel and forceps with dH_20 followed by **Ethanol.**
4. Cut adult thorax in half and add to tube, or add whole larva.
5. Wash instruments as step 3.
6. Repeat step 4 for all samples, avoiding cross-contamination

7. Wash micropestle with dH_20 followed by ethanol. Grind sample in tube with micropestle.
8. Repeat step 7 for all samples, avoiding cross-contamination.
9. Spin in picofuge briefly to collect samples at bottom of tube.
10. Add **150 µl Grinding Buffer.** Mix well and incubate at 65°C for 30 minutes.
11. While tubes are still warm, add **43 µl 8M KAc.** Mix well, incubate on wet ice for 30 minutes to precipitate salt and SDS.
12. Centrifuge at 14,000xg for **15 minutes**.
13. Label a new set of tubes.
14. Transfer supernatants to new tubes.
15. Add **250 µl Ice Cold Ethanol**, mix well and place tubes in -80°C freezer for 1 hour or overnight at -20°C.
16. Remove from freezer and centrifuge at 14,000xg for **15 minutes**.
17. Remove supernatant and allow to dry.
18. Resuspend pellet in 25 µl ddH_2O.
19. Assess success of extractions by running 2 µl extract on an agarose gel.

8.3.c Performing PCR

8.3.c.i. PCR reaction master mix:

- Primers (1.25 micromolar = "Everyday" primer solution)
- dNTP (5mM, made by 1:1 dilution of stock 10mM dNTP's with ddH2O)
- NH4 Buffer (10x stock solution)
- Taq (5U / microlitre stock solution)
- MgCl2 (25mM stock solution)
- Template DNA (10 fold dilution of original DNA extraction with ddH2O)
- ddH2O

8.3.c.ii. Method:

8.3.c.iii. In non-PCR lab:

1. Take the required DNA extractions from the -80°C freezer.
2. Label **0.5ml** tubes with sample numbers.
3. Pipette **2 µl** of diluted DNA into each of the designated tubes. AVOID CROSS CONTAMINATION.
4. Place these tubes into the fridge whilst the PCR MasterMix is being prepared.
5. In a **1.5ml** tube, add the NH_4, dNTPs, $MgCl_2$, Primer Mix ("Everyday") and ddH_2O, and mix. Place this tube into the fridge.
6. Collect together the Auto-pipette, Pasteur pipette, Mineral oil and the 5-40 µl pipette and place on a tray.
7. Take the PCR mixture from the fridge and add the **Taq**. Return the Taq back to the fridge straight away, to stop the Taq enzyme degrading in the light. Taq will start working in the mix straight away so the rest of the procedure **MUST** be done as quickly as possible!
8. Take the prepared tubes containing the samples from the fridge and put on the tray with the other equipment and proceed to the PCR lab.

8.3.c.iv. In the PCR lab:

1. Ensure all the sample tubes are open.
2. Using the auto-pipette, draw the PCR MasterMix up by pressing the button **underneath** the pipette. The auto-pipette is set to deliver 8µl so does not need to be altered.
3. Add MasterMix to each of the sample tubes by simply pressing the underneath button **once** over each of the tubes.
4. The auto-pipette needs to be reset after every 12 tubes (i.e. each row). Make sure to hold the pipette tip over the MasterMix tube before pressing the reset button.
5. Using the Pasteur pipette, add 2 drops of mineral oil to each of the tubes.
6. Cap the tubes and load the PCR machine.

8.3.c.v. PCR Machine:

The program cycle is as follows:

- Initially : 94°c 4 minutes, 54°c 1 minute, 72°c 45 seconds, 1 cycle.
- Then 30 cycles: 92°c 1 minute, 54°c 1 minute, 72°c 45 seconds.
- Finally: 72 °c 10 minutes.

8.3.d Preparing PCR products for sequencing

- Prepare a 1:90 dilution of ROX size standard with Formamide.
- To each well of the 96-well plate, add 10 µl of the above mixture.
- Add 1.1 µl PCR product to each well.
- Cover wells with well caps.
- Mix (tapping plate lightly on desk/ rubbing spines across surface).
- Centrifuge for 1 minute at 1000rpm.
- Denature at 95°c for 5 minutes then remove **immediately** and put on ice for 5 minutes.
- Sequence immediately in ABI 3100 sequencer or store at -80°c until needed.

8.4 Appendix 4: Larval feeding data

Nest	Year	Larval size	Weight (g)	Larval species/nest: Control (A)/ Host on parasitised nest (B)/ Parasite (C)	Group size	No. Cells	Relative day filmed	No. larvae	Total feed before	Total feed after
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	1	0
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	0	1
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	0	0
7	2004	1	N/A	A	3	61	18	17	1	0
7	2004	1	N/A	A	3	61	18	17	0	1
7	2004	2	N/A	A	3	61	18	17	0	0
7	2004	2	N/A	A	3	61	18	17	0	2
7	2004	2	N/A	A	3	61	18	17	2	3
7	2004	2	N/A	A	3	61	18	17	0	2
7	2004	2	N/A	A	3	61	18	17	2	1
7	2004	2	N/A	A	3	61	18	17	1	3
7	2004	2	N/A	A	3	61	18	17	1	1
16	2004	1	N/A	B	3	138	43	46	0	1

16	2004	1	N/A	B	3	138	43	46	2	1
16	2004	2	N/A	B	3	138	43	46	4	0
16	2004	2	N/A	B	3	138	43	46	0	1
16	2004	2	N/A	B	3	138	43	46	2	0
16	2004	2	N/A	B	3	138	43	46	1	3
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	1	0
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	2	2
16	2004	1	N/A	C	3	138	43	46	3	0
16	2004	1	N/A	C	3	138	43	46	1	0
16	2004	1	N/A	C	3	138	43	46	2	0
16	2004	1	N/A	C	3	138	43	46	0	0
16	2004	1	N/A	C	3	138	43	46	2	2
16	2004	1	N/A	C	3	138	43	46	1	1
16	2004	2	N/A	C	3	138	43	46	2	0
16	2004	2	N/A	C	3	138	43	46	1	1
16	2004	2	N/A	C	3	138	43	46	4	4
16	2004	2	N/A	C	3	138	43	46	1	4
16	2004	2	N/A	C	3	138	43	46	1	1
16	2004	2	N/A	C	3	138	43	46	1	2
45	2005	1	N/A	A	4	141	16	12	0	0
45	2005	1	4E-04	A	4	141	16	12	0	0
45	2005	1	0.004	A	4	141	16	12	0	0
45	2005	1	0.005	A	4	141	16	12	0	0

45	2005	1	0.007	A	4	141	16	12	2	0
45	2005	1	0.008	A	4	141	16	12	0	0
45	2005	1	0.01	A	4	141	16	12	0	2
45	2005	1	0.043	A	4	141	16	12	0	1
45	2005	1	0.057	A	4	141	16	12	1	2
45	2005	1	0.058	A	4	141	16	12	0	0
45	2005	2	0.121	A	4	141	16	12	1	2
45	2005	2	0.164	A	4	141	16	12	3	6
62	2005	1	N/A	C	4	155	16	41	0	0
62	2005	1	4E-04	C	4	155	16	41	0	0
62	2005	1	0.004	C	4	155	16	41	0	0
62	2005	1	0.005	C	4	155	16	41	0	0
62	2005	1	0.007	C	4	155	16	41	2	0
62	2005	1	0.008	C	4	155	16	41	0	0
62	2005	1	0.01	C	4	155	16	41	0	2
62	2005	1	0.043	C	4	155	16	41	0	1
62	2005	1	0.057	C	4	155	16	41	1	2
62	2005	1	0.058	B	4	155	16	41	0	0
62	2005	2	0.121	B	4	155	16	41	1	6
62	2005	2	0.164	C	4	155	16	41	3	6
64	2004	1	N/A	A	3	64	40	11	1	0
64	2004	2	N/A	A	3	64	40	11	3	1
64	2004	2	N/A	A	3	64	40	11	2	1
64	2004	2	N/A	A	3	64	40	11	1	1
81	2004	1	N/A	A	3	98	45	36	0	0
81	2004	1	N/A	A	3	98	45	36	0	0
81	2004	1	N/A	A	3	98	45	36	0	4
81	2004	1	N/A	A	3	98	45	36	0	2

81	2004	1 N/A	A	3	98	45	36	1	1
81	2004	1 N/A	A	3	98	45	36	1	2
81	2004	1 N/A	A	3	98	45	36	1	1
81	2004	1 N/A	A	3	98	45	36	5	1
81	2004	1 N/A	A	3	98	45	36	1	1
81	2004	1 N/A	A	3	98	45	36	0	0
81	2004	1 N/A	A	3	98	45	36	0	2
81	2004	1 N/A	A	3	98	45	36	1	4
81	2004	1 N/A	A	3	98	45	36	2	2
81	2004	1 N/A	A	3	98	45	36	2	1
81	2004	1 N/A	A	3	98	45	36	1	1
81	2004	2 N/A	A	3	98	45	36	4	2
81	2004	2 N/A	A	3	98	45	36	3	2
81	2004	2 N/A	A	3	98	45	36	1	1
81	2004	2 N/A	A	3	98	45	36	4	3
81	2004	2 N/A	A	3	98	45	36	2	3
81	2004	2 N/A	A	3	98	45	36	2	3
81	2004	2 N/A	A	3	98	45	36	0	5
81	2004	2 N/A	A	3	98	45	36	2	1
81	2004	2 N/A	A	3	98	45	36	3	1
81	2004	2 N/A	A	3	98	45	36	1	5
81	2004	2 N/A	A	3	98	45	36	3	3
81	2004	2 N/A	A	3	98	45	36	4	5
81	2004	2 N/A	A	3	98	45	36	2	0
81	2004	2 N/A	A	3	98	45	36	3	4
81	2004	2 N/A	A	3	98	45	36	2	1
81	2004	2 N/A	A	3	98	45	36	5	2
81	2004	2 N/A	A	3	98	45	36	1	2

81	2004	2	N/A	A	3	98	45	36	4	3
81	2004	2	N/A	A	3	98	45	36	4	2
81	2004	2	N/A	A	3	98	45	36	4	5
81	2004	2	N/A	A	3	98	45	36	6	5
134	2005	1	0.008	A	2	96	23	14	0	0
134	2005	1	0.012	A	2	96	23	14	1	0
134	2005	1	0.016	A	2	96	23	14	2	0
134	2005	1	0.03	A	2	96	23	14	1	1
134	2005	1	0.036	A	2	96	23	14	1	0
134	2005	1	0.039	A	2	96	23	14	0	0
134	2005	1	0.047	A	2	96	23	14	3	6
134	2005	1	0.049	A	2	96	23	14	3	0
134	2005	1	0.058	A	2	96	23	14	1	2
134	2005	1	0.067	A	2	96	23	14	1	2
134	2005	2	0.077	A	2	96	23	14	3	0
134	2005	2	0.092	A	2	96	23	14	1	2
134	2005	2	0.154	A	2	96	23	14	2	1
134	2005	2	0.158	A	2	96	23	14	2	1
165	2005	1	N/A	C	8	132	13	17	0	0
165	2005	1	N/A	C	8	132	13	17	0	0
165	2005	1	N/A	C	8	132	13	17	0	0
165	2005	1	0.01	C	8	132	13	17	0	0
165	2005	1	0.018	C	8	132	13	17	0	0
165	2005	1	0.021	C	8	132	13	17	0	0
165	2005	1	0.043	C	8	132	13	17	0	0
165	2005	2	0.048	C	8	132	13	17	1	0
165	2005	2	0.072	C	8	132	13	17	4	7
165	2005	2	0.094	C	8	132	13	17	1	4

165	2005	2	0.102	C	8	132	13	17	1	3
165	2005	2	0.107	C	8	132	13	17	1	1
165	2005	2	0.113	B	8	132	13	17	1	0
165	2005	2	0.152	C	8	132	13	17	1	2
170	2005	1	N/A	C	3	73	6	15	0	0
170	2005	1	0.004	C	3	73	6	15	0	0
170	2005	1	0.004	C	3	73	6	15	3	0
170	2005	1	0.005	C	3	73	6	15	0	0
170	2005	1	0.008	C	3	73	6	15	0	0
170	2005	1	0.012	C	3	73	6	15	0	0
170	2005	1	0.012	C	3	73	6	15	0	0
170	2005	1	0.025	C	3	73	6	15	0	0
170	2005	1	0.056	B	3	73	6	15	2	0
170	2005	1	0.066	C	3	73	6	15	0	0
170	2005	2	0.072	B	3	73	6	15	1	0
170	2005	1	0.084	B	3	73	6	15	6	0
170	2005	2	0.091	B	3	73	6	15	2	0
170	2005	1	0.102	B	3	73	6	15	5	0
172	2005	1	0.008	C	2	73	4	15	0	0
172	2005	1	0.012	C	2	73	4	15	1	0
172	2005	1	0.016	C	2	73	4	15	0	0
172	2005	1	0.03	C	2	73	4	15	1	1
172	2005	1	0.036	C	2	73	4	15	1	0
172	2005	1	0.039	C	2	73	4	15	0	0
172	2005	1	0.047	C	2	73	4	15	0	0
172	2005	1	0.049	C	2	73	4	15	1	2
172	2005	1	0.058	C	2	73	4	15	1	2
172	2005	1	0.067	C	2	73	4	15	2	0

172	2005	2	0.077	B	2	73	4	15	3	0
172	2005	2	0.092	C	2	73	4	15	1	2
172	2005	2	0.154	C	2	73	4	15	2	1
172	2005	2	0.158	B	2	73	4	15	2	1
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	1	1
48a	2005	1	N/A	A	4	50	15	15	1	1
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	1	N/A	A	4	50	15	15	2	1
48a	2005	1	N/A	A	4	50	15	15	2	2
48a	2005	1	N/A	A	4	50	15	15	1	1
48a	2005	1	N/A	A	4	50	15	15	0	0
48a	2005	2	N/A	A	4	50	15	15	0	0
48a	2005	2	N/A	A	4	50	15	15	2	1
48a	2005	2	N/A	A	4	50	15	15	2	1
58a	2005	1	N/A	A	4	56	9	26	0	0
58a	2005	1	N/A	A	4	56	9	26	4	0
58a	2005	1	N/A	A	4	56	9	26	1	0
58a	2005	1	N/A	A	4	56	9	26	1	0
58a	2005	1	N/A	A	4	56	9	26	1	0
58a	2005	1	N/A	A	4	56	9	26	1	0
58a	2005	1	N/A	A	4	56	9	26	0	2
58a	2005	1	N/A	A	4	56	9	26	0	3
58a	2005	1	N/A	A	4	56	9	26	2	1

58a	2005	1 N/A	A	4	56	9	26	4	0
58a	2005	1 N/A	A	4	56	9	26	10	5
58a	2005	1 N/A	A	4	56	9	26	5	0
58a	2005	1 N/A	A	4	56	9	26	5	1
58a	2005	1 N/A	A	4	56	9	26	3	0
58a	2005	1 N/A	A	4	56	9	26	3	0
58a	2005	1 N/A	A	4	56	9	26	3	3
58a	2005	2 N/A	A	4	56	9	26	3	0
58a	2005	2 N/A	A	4	56	9	26	5	3
58a	2005	2 N/A	A	4	56	9	26	0	0
58a	2005	2 N/A	A	4	56	9	26	0	0
58a	2005	2 N/A	A	4	56	9	26	8	1
58a	2005	2 N/A	A	4	56	9	26	3	1
58a	2005	2 N/A	A	4	56	9	26	7	0
58a	2005	2 N/A	A	4	56	9	26	4	2
58a	2005	2 N/A	A	4	56	9	26	5	0
58a	2005	2 N/A	A	4	56	9	26	4	5
nr121	2004	2 N/A	B	2	99	45	33	0	1
nr121	2004	2 N/A	B	2	99	45	33	5	6
nr121	2004	2 N/A	B	2	99	45	33	5	3
nr121	2004	2 N/A	B	2	99	45	33	1	2
nr121	2004	1 N/A	C	2	99	45	33	0	0
nr121	2004	1 N/A	C	2	99	45	33	0	0
nr121	2004	1 N/A	C	2	99	45	33	0	1
nr121	2004	1 N/A	C	2	99	45	33	0	0
nr121	2004	2 N/A	C	2	99	45	33	1	2
nr121	2004	2 N/A	C	2	99	45	33	2	1
nr59a	2004	1 N/A	A	3	61	47	23	0	0

nr59a	2004	1 N/A	A	3	61	47	23	0	1
nr59a	2004	1 N/A	A	3	61	47	23	0	0
nr59a	2004	1 N/A	A	3	61	47	23	0	0
nr59a	2004	1 N/A	A	3	61	47	23	0	0
nr59a	2004	1 N/A	A	3	61	47	23	1	0
nr59a	2004	1 N/A	A	3	61	47	23	0	3
nr59a	2004	1 N/A	A	3	61	47	23	3	0
nr59a	2004	1 N/A	A	3	61	47	23	2	5
nr59a	2004	1 N/A	A	3	61	47	23	1	0
nr59a	2004	1 N/A	A	3	61	47	23	4	3
nr59a	2004	1 N/A	A	3	61	47	23	1	5
nr59a	2004	2 N/A	A	3	61	47	23	2	6
nr59a	2004	2 N/A	A	3	61	47	23	0	6
nr59a	2004	2 N/A	A	3	61	47	23	3	7
nr59a	2004	2 N/A	A	3	61	47	23	2	13
nr59a	2004	2 N/A	A	3	61	47	23	4	10
nr59a	2004	2 N/A	A	3	61	47	23	3	5
nr59a	2004	2 N/A	A	3	61	47	23	6	7
nr59a	2004	2 N/A	A	3	61	47	23	2	14
nr59a	2004	2 N/A	A	3	61	47	23	1	0
nr59a	2004	2 N/A	A	3	61	47	23	0	1
nr59a	2004	2 N/A	A	3	61	47	23	2	12
nrp4	2004	1 N/A	B	2	84	45	30	0	3
nrp4	2004	1 N/A	B	2	84	45	30	0	2
nrp4	2004	1 N/A	B	2	84	45	30	0	2
nrp4	2004	2 N/A	B	2	84	45	30	5	1
nrp4	2004	2 N/A	B	2	84	45	30	0	3
nrp4	2004	1 N/A	C	2	84	45	30	0	0

nrp4	2004	1	N/A	C	2	84	45	30	4	1
nrp4	2004	1	N/A	C	2	84	45	30	0	0
nrp4	2004	1	N/A	C	2	84	45	30	1	0
nrp4	2004	1	N/A	C	2	84	45	30	0	0
nrp4	2004	1	N/A	C	2	84	45	30	1	1
nrp4	2004	1	N/A	C	2	84	45	30	2	1
nrp4	2004	1	N/A	C	2	84	45	30	0	1
nrp4	2004	2	N/A	C	2	84	45	30	0	5
nrp4	2004	2	N/A	C	2	84	45	30	0	1
nrp4	2004	2	N/A	C	2	84	45	30	0	3
nrp4	2004	2	N/A	C	2	84	45	30	2	3
nrp4	2004	2	N/A	C	2	84	45	30	0	1
nrp4	2004	2	N/A	C	2	84	45	30	1	2
nrp4	2004	2	N/A	C	2	84	45	30	1	2
nrp4	2004	2	N/A	C	2	84	45	30	0	5
nrp4a	2004	1	N/A	A	4	55	45	11	0	1
nrp4a	2004	1	N/A	A	4	55	45	11	0	1
nrp4a	2004	1	N/A	A	4	55	45	11	1	0
nrp4a	2004	1	N/A	A	4	55	45	11	2	1
nrp4a	2004	1	N/A	A	4	55	45	11	2	2
nrp4a	2004	1	N/A	A	4	55	45	11	1	0
nrp4a	2004	1	N/A	A	4	55	45	11	0	0
nrp4a	2004	1	N/A	A	4	55	45	11	2	2
nrp4a	2004	2	N/A	A	4	55	45	11	1	1
nrp4a	2004	2	N/A	A	4	55	45	11	2	3
nrp4a	2004	2	N/A	A	4	55	45	11	3	3
s16	2005	1	0.003	C	5	81	0	25	11	7
s16	2005	1	0.007	C	5	81	0	25	0	0

s16	2005	1	0.007 B	5	81	0	25	1	0
s16	2005	1	0.008 B	5	81	0	25	0	0
s16	2005	1	0.009 C	5	81	0	25	0	0
s16	2005	1	0.011 C	5	81	0	25	8	9
s16	2005	1	0.018 B	5	81	0	25	0	1
s16	2005	1	0.02 B	5	81	0	25	0	0
s16	2005	1	0.033 C	5	81	0	25	0	1
s16	2005	1	0.04 C	5	81	0	25	0	7
s16	2005	1	0.065 B	5	81	0	25	3	4
s16	2005	1	0.065 B	5	81	0	25	0	0
s16	2005	1	0.071 B	5	81	0	25	0	0
s16	2005	1	0.078 B	5	81	0	25	13	0
s16	2005	1	0.085 C	5	81	0	25	10	6
s16	2005	2	0.113 B	5	81	0	25	17	3
s16	2005	2	0.121 C	5	81	0	25	7	3
s16	2005	2	0.139 C	5	81	0	25	15	6
s16	2005	2	0.17 C	5	81	0	25	4	1
s172	2005	1	0.005 C	2	55	4	9	14	11
s172	2005	1	0.005 C	2	55	4	9	18	3
s172	2005	1	0.006 C	2	55	4	9	3	1
s172	2005	1	0.054 C	2	55	4	9	1	7
s172	2005	2	0.102 B	2	55	4	9	10	7
s172	2005	2	0.105 C	2	55	4	9	0	1

8.5 Appendix 5 – Molecular Data

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
62	GYBPi	A	132				194	211	258	258	151	151
62	GRYPi	A					194	211	255	255	151	151
62	w (white)	A	127	146			200				148	151
62	Parasit e PiPi	A	127	153	187	187	212	225	261	264	173	184
62	RYPi	A									148	151
62	1.08	B	127	127	187	187	222	222	261	261	184	184
62	1.09	B	-	-	-	-	222	231	261	264	154	173
62	3.25	B	127	127							154	173
62	3.39	B		127			211		258		151	
62	1.45	B	127	127			225	225	264	264	184	184
62	1.46	B	127	163	187	215	225	231	255	264	154	173
62	1.58	B	127	127	187	187	225	225	264	264	184	184
62	1.72	B	127	163	-	-	212	231	255	261	154	173
62	1.102	B	127	127	187	187	225	225	264	264	184	184
62	1.133	B	115	146	-	-	206	212	225	258	148	148
62	2.134	B	153	153	-	-	225	225	264	264	173	173

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
165	BBBPi	A	113	138	193	199	206	236	217	253	151	154
165	"- RRPi"	A	113	123	193	199	212	236	217	253	151	154
165	w-wPi	A	113	138	193	199	205	236	217	217	151	154
165	WGW Pi	A	113	123	193	199	211	236	217	217	154	154
165	RWRP i	A	113	123	186	192	209	233	214	250	154	154
165	RYRPi	A	113	123	192	198	209	235	215	253	151	154
165	Parasit e –R	A	127	139	200	216	213	228	261	264	154	157
165	"-BB-"	A	113	139	193	200	217	217	206	236	151	154
165	WBWP i	A	113	123	193	199	211	236	215	215	151	154
165	1.34	B	139	139	-	-	213	213	261	261	154	154
165	3.42	B	130	139	186	200	223	228	256	261	154	157
165	3.54	B	127	130	186	216	213	223	256	261	154	157
165	3.57	B	130	139	-	-	223	228	256	261	154	157
165	1.64	B	127	130	186	200	213	223	256	264	154	157
165	1.67	B	127				228		261		157	

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
165	3.76	B	113	113	192	192	209	-	-	-	154	154
165	1.77	B	127	127	-	-	228	228		-	157	-
165	3.78	B	139		216		213		261	-	157	
165	3.84	B	127	130	186	200	223	228	256	264	154	154
165	1.90	B	139				228		264		154	
165	1.103	B	127	127	200	200	228	228	264	264	154	154
165	1.104	B	127	127							154	154
165	3.113	B	127	127	216	216	213	213	261	261	154	154
165	2.115	B	127	127	216	216	228	228	261	261	154	154

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
170	Parasit eRP	A	123	129	215	221	206	212	248	291	154	157
170	1.06	B	130	147			212		248	257	157	
170	3.10	B	137	140			203	212	215	215	151	157
170	3.16	B	137	140			203	212	215	215	154	157
170	1.21	B	129	129	-	-	206	206	-	-	154	154
170	1.23	B	137	140							154	157
170	2.36	B	140	162	192	192	203	203	215	215	151	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
170	2.43	B	137	140			203	203			151	157
170	2.51	B	140	162	192	192	203	203	215	215	154	157
170	1.56	B	125	125	223?		206	206	250	250	157	157
170	1.58	B	130	147	223		212	212	250	258	157	157
170	1.59	B	124	124			206	206			154	154
170	1.60	B	124	147	221		212	212	258	291	157	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
s16	"—R-"	A	126	138	192	204	206	209	255	258	148	151
s16	PiRYR	A	126	138	192	192	206	209	252	258	151	157
s16	PiRYY	A	126	138	192	192	206	209	255	258	151	157
s16	"- BGW"	A	126	138	-	-	206	209	255	261	151	157
s16	PiBWY	A	126	135	192	204	206	209	255	258	148	151
s16	Parasit eRR	A	111	126	-	-	212	221	261	282	154	160
s16	2.16	B	126	135	204	204	206	212	255	261	151	154
s16	1.17	B	126	135			206	212	261	261	151	154
s16	3.19	B	126	135	192	192	212	215	261	261	151	154
s16	1.21	B	126	135	189	192	206	221	255	261	151	154
s16	2.25	B	138	138	192	192	209	209	252	252	151	151
s16	1.31	B	126	135	192	192	206	212	255	261	151	154
s16	2.38	B	126	138	192	204	203	206	238	255	148	157
s16	2.40	B	126		189	192	206	215			154	154
s16	3.45	B	126				212	206	255	261	151	160
s16	1.47	B					206	212	255	255	154	157
s16	2.48	B	126	126	192	192	206	206	255	255	148	148

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
s16	2.53	B	126	138	192	204	203	206	238	255	148	160
s16	3.59	B					206	209	255	255	154	157
s16	1.60	B					206	209	255	255		
s16	1.62	B					206	209	255	255		
s16	2.63	B	126	135	-	-	206	212	258	258	151	154
s16	2.64	B	132	138			206	212	252	255	151	154
s16	1.71	B	126	135	204	204	206	212	255	261	151	160

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
s172	Parasi teBR	A	126	138	192	195	213	219	255	258	157	157
s172	GBW Pi	A	135	144			203	206	255	255	151	151
s172	RGR Pi	A	117	141	180	186	203	206	216	231	148	148
s172	2.06	B	126	126	192	192	213	213	258	258	157	157
s172	2.07	B	138	138	192	192	213	213	258	258	157	157
s172	3.14	B	144	144							151	151
s172	1.26	B	125	138			221	213	258	258	157	179

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
s172	1.30	B	126	138			219	221	258	258	157	178
s172	1.43	B	126	138					258	258	157	178

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
172N	Parasi teYW	A	139	139	189	192	239	213	266	257	157	175
172N	RGG Pi	A	133	139	186	200	203	209	2522 38	252	151	151
172N	3.07	B	133	139	-	-	-	-	-	-	151	157
172N	3.08	B	133	139	192	192	200	206	252	252	151	157
172N	3.16	B	133	-	189	-	218	239	257	266	153	157
172N	2.22	B	139	139	189	189	218	239	257	266	157	175
172N	1.27	B							257	266	153	157
172N	2.32	B	133		189	192	218	239	257	266	153	157
172N	2.34	B					229	238	257	266	153	157
172N	2.38	B	139	139	189	189	218	218	255	255	175	175
172N	1.79	B					218	218			154	157
172N	2.74	B	139				218	239	257	266	157	175
172N	3.73	B	133	139	186	201	203	209	238		151	151

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
172N	2.88	B					229	238	257	266	157	175
172N	2.89	B	133		189	192	219	240	258	266	157	175
172N	1.94	B	139	139	189		219	239	257	266	154	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdom 7
16	ParasiteYW	A	126	129	192	213	218	218	255	255	157	157
16	-PBY	A	108	138	192	204	194	200	261	261	148	151
16	PRYG	A	108	138	192	198	194	200	261	261	148	151
16	PWYY	A	108	132	192	204	194	203	261	261	148	151
16	0.1	B	126	151	212	212	212	218	252	255	148	151
16	0.106	B	129	151	213	213	212	218	255	258	154	157
16	0.13	B	129	151	192	213	212	218	255	258	154	157
16	0.14	B	126	138	-	-	194	203	-	-	151	151
16	0.19	B	129	151	192	213	212	218	255	258	154	157
16	0.2	B	126	138	-	-	194	203	-	-	151	151
16	0.3	B	129	151	192	213	212	218	255	258	154	157
16	0.38	B	126	151	192	213	212	218	255	258	154	157
16	0.38	B	129	129	213	213	212	218	255	255	157	157
16	0.41	B	126	151	192	213	212	218	255	258	154	157
16	0.44	B	129	129	213	213	218	218	255	255	157	157
16	0.53	B	129	151	213	213	212	218	255	258	154	157
16	0.56	B	129	151	192	213	212	218	255	258	154	157
16	0.59	B	126	151	213	213	212	218	255	258	154	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
16	0.6	B	108	126	192	198	194	203	255	255	148	151
16	0.61	B	126	135	-	-	215	218	-	-	154	169
16	0.62	B	126	126	213	213	218	218	255	255	157	157
16	0.66	B	126	151	213	213	212	218	255	258	154	157
16	45	B	129	151	213	213	212	218	255	258	154	157
16	78	B	129	151	213	213	212	218	255	258	154	157
16	81	B	129	151	213	213	212	218	255	258	154	157
16	84	B	108	126	192	198	194	203	255	255	148	151
16	86	B	126	126	192	192	218	218	255	255	157	157
16	91	B	126	126	213	213	218	218	255	255	157	157
16	96	B	126	138	192	198	200	203	-	-	148	151
16	97	B	129	151	213	213	212	218	255	258	154	157
16	104	B	129	129	213	213	218	218	255	255	157	157
16	105	B	129	129	192	192	218	218	255	255	157	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
n121	BRPR	A	126	139	192	192	200	212	255	258	148	151
n121	RRP G	A	139	142	192	198	206	212	216	258	151	151
n121	Parasi teYR	A	125	151	192	219	222	222	249	249	158	181
n121	0.3	B	127	146			201	213	231	255	148	151
n121	0.65	B	127	127	192	198	200	212	230	255	142	151
n121	0.68	B	127	127	192	198	200	212	230	255	142	151
n121	0.77	B	125		216		222		249			181
n121	0.66	B	139	139	181	181	212	212	255	255	151	151
n121	0.55	B	137	151	216	226	222	222	249	249	158	181
n121	0.54	B	137	151	216	226	222	222	249	249	158	181
n121	0.19	B	125	137	192	226	222	222	249	249	158	181
n121	0.73	B	151		192		222	222	249	249	181	181

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
nrp4	BBPW	A	127	133	192	192	200	206	216	216	148	151
nrp4	BrBrPi Br	A	127	133	192	192	209	209	216	216	157	163
nrP4	Parasit eYY	A	122	125	210	213	212	215	257	263	154	181
nrP4	0.12	B	122	134	198	-	215	218	252	264	170	182
nrP4	0.13	B	124	133	198	209	211	216	262	249	153	169
nrP4	0.24	B	121	133	197	212	211	216	249	256	169	180
nrP4	0.29	B	122	134	198	214	216	219	255	261	169	181
nrP4	0.3	B	122	134	199	210	216	219	252	258	171	181
nrP4	0.38	B	122	134	198	-	216	220	255	266	155	170
nrP4	0.4	B	122	-		-	212	-	264	-	154	-
nrP4	0.51	B	122				216		258		155	
nrP4	0.54	B	122	134	199	214	215	218	252	264	169	181
nrP4	0.55	B	122	134	199	-	212	218	252	264	170	182
nrP4	0.56	B	-	-	-	-	198	209	-	-	142	151
nrP4	0.57	B	125	134	198	211	216	220	255	261	169	181
nrP4	0.66	B	125	134	198	214	216	219	252	263	154	169
nrP4	0.69	B	127	133	180	186	209	233	214	294	151	157

Nest	Id	(A)dult/ (B)rood	Pdom 127	Pdom 127	Pdom 139	Pdom 139	Pdom 140	Pdom 140	Pdom 20	Pdom 20	Pdom 7	Pdo m 7
nrP4	0.76	B	112	133	180	186	209	233	216	294	151	157
nrP4	58	B	122	134	199	212	213	219	252	263	154	169
nrP4	59	B	112	132	186	189	209	233	216	294	157	157
nrP4	61	B	112	133	-	-	200	233	216	294	148	157
nrP4	65	B	122	134	199	215	213	220	255	261	169	181
nrP4	67	B	122	134	198	212	215	218	252	263	169	181